Human Papillomavirus Can Escape Immune Recognition through Langerhans Cell Phosphoinositide 3-Kinase Activation

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Human papillomavirus (HPV) infection of cervical epithelium is linked to the generation of cervical cancer in women (1). Currently there are no therapeutic or prophylactic vaccine strategies available and HPV cannot be easily produced in vitro, which hinders vaccine development. HPV virus-like particles (VLP) composed of the L1 viral capsid protein are being explored as a vaccination strategy to prevent HPV infection, with promising results in clinical trials (2). HPV VLP initiate neutralizing Abs capable of preventing HPV infection in vaccine recipients indicating that HPV VLP have similar antigenic determinants as native HPV capsid. Therefore, HPV VLP serve as a model to determine how HPV interacts with cells of the immune system. Knowledge of how HPV interacts with cells of the immune system will aid in our understanding of HPV immunosurveillance and HPV vaccine development.

The importance of the immune system in the control of HPV infection and lesion development is shown by the fact that increased cellular immune responses correlate with a good clinical prognosis (3). In addition, immunocompromised patients exhibit more persistent infection and an increase in their number of lesion recurrences (4, 5). Although the majority of virally induced lesions are cleared, the time for clearance ranges from months to years. Some women do not even initiate an effective immune response against HPV indicating that the virus has evolved mechanisms to evade host immunity (3, 6). Various direct and indirect mechanisms used by HPV to evade host immunity have been previously described (7). However, no mechanism has been identified in which HPV directly alters immune system activation via its interaction with the professional APC, Langerhans cells (LC), at the site of infection.

After pathogen encounter, APC may initiate various multiple signal transduction pathways culminating in expression of a variety of different immune response genes (8–10). A pathogen can activate various members of the MAPK family, the NF-κB cascade, and/or the phosphoinositide 3-kinase (PI3-K) cascade to induce a response (8–10). The expression profile initiated is specific to the particular Ag resulting in a response unique to that Ag. Therefore various pathogens have evolved mechanisms to induce or inhibit particular signaling cascades to aid in their escape from host immunity (11, 12).

We previously found that LC incubated with HPV VLP did not up-regulate surface activation markers, did not increase secretion of proinflammatory cytokines, and did not initiate an epitope-specific immune response against VLP-derived Ags, whereas dendritic cells (DC), a professional APC targeted by vaccination procedures, did initiate a response (13). Furthermore, data indicate that LC cross-presented HPV VLP-derived peptides on their surface, but they did so in the absence of costimulation rendering the LC potentially immunosuppressive (14). In the current study we determined the intracellular signaling cascades initiated in human DC and LC after HPV VLP encounter. After HPV VLP stimulation, the MAPK, NF-κB, and PI3-K signaling cascades are initiated in DC culminating in cellular activation. However in LC, the PI3-K signaling cascade is activated whereas the MAPK pathways are reduced after the LC encounter with HPV VLP. Furthermore, the up-regulation of PI3-K in LC led to the inactivation of Akt, which was mediated through serine/threonine protein phosphatase 2A (PP2A). After inhibition of the PI3-K cascade, LC up-regulated surface activation markers and induced a potent immune response against HPV VLP-derived Ags. Taken together these data indicate...
that the lack of ability of LC to induce an anti-HPV immune response is due to the activation of PI3-K and defines a novel immune escape mechanism used by HPV.

**Materials and Methods**

**VLP production**

HPV16-L1L2 VLP and HPV16-L1L2-E7 VLP were produced in insect cells and purified by sucrose and cesium chloride ultracentrifugation as previously described (13). A Limulus assay (Sigma-Aldrich) was used to detect and semiquantitate endotoxin in the preparations. The amount of endotoxin detected was 0.085 endotoxin units (EU)/10 μg of HPV VLP and this level did not activate DC or LC (data not shown). Bacterial DNA used in VLP production procedure did not activate either DC or LC (data not shown).

**Donor material**

PBL from healthy donors were obtained by leukapheresis. Leukocytes were purified by Ficoll gradient centrifugation (Nycomed) and stored in liquid nitrogen. HPV serology analysis of all donors showed negative results, indicating no prior exposure to the virus.

**DC and LC generation**

DC and LC were generated as previously described (13). Briefly, frozen PBL were thawed and washed once with RPMI 1640, containing 10 mM sodium pyruvate (Invitrogen Life Technologies), 10 mM nonessential amino acids (Invitrogen Life Technologies), 100 μg/ml kanamycin (Sigma-Aldrich), and 10% FCS (HyClone Laboratories). For DC, plastic adherent cells were selected by plating 200 × 10^6 cells in a 175 cm² tissue culture flask for 2 h at 37°C. Nonadherent cells were washed away and the remaining adherent cells were cultured for 6 days in medium containing 1000 U/ml recombinant human GM-CSF (Intergen) and 1200 U/ml recombinant human IL-4 (Intergen) of which 50% was replenished every other day. For LC, adherent cells were cultured for 6 days in medium containing 1000 U/ml recombinant human GM-CSF, 1200 U/ml recombinant human IL-4, and 10 ng/ml recombinant human TGF-β1 (PeproTech) of which 50% was replenished every other day.

**Activation assay**

DC and LC were collected, washed twice with PBS, and either left untreated or treated with 10 μM MAPK inhibitor SB203580 (Sigma-Aldrich), 10 μM NF-κB inhibitor BAY11-7082 (Biomol), or 20 μM PI3-K inhibitor LY294002 (EMD Biosciences) for 30 min at 37°C. DC and LC were subsequently incubated with or without 10 μg of LPS (Sigma-Aldrich) or 10 μg of HPV16-L1L2 VLP/10^6 cells for 1 h at 37°C. Cells were then incubated for 48 h in 15 ml of complete medium containing 1000 U/ml recombinant human GM-CSF. Cells were harvested, washed, and stained for flow cytometric analysis. Cells were stained for MHC class I (DAKO), CD80, CD86, and isotype controls (BD Pharmingen).

**Western blot**

DC and LC were collected, washed twice with PBS, and either left untreated or treated with 20 μM LY294002, 100 ng/ml PMA (Calbiochem), or 500 nM okadaic acid (OA; Calbiochem) for 30 min at 37°C. Subsequently the DC or LC were incubated with or without 10 μg of LPS or 10 μg of HPV16-L1L2 VLP/10^6 cells at 37°C for the times indicated. Cellular extracts were prepared using the Mammalian Protein Extraction Reagent (Pierce). Immunoblotting of cellular extracts was performed using Abs to phosphorylated ERK1/2, ERK1/2, phosphorylated MAPK kinase (MKK4), MKK4, phosphorylated activating transcription factor (ATF2), ATF2, IκBα, phosphorylated PI3-K, PI3-K, phosphorylated Akt, Akt (Santa Cruz Biotechnology), or GAPDH (Chemicon International). Secondary Abs were anti-goat IgG HRP (Vector Laboratories) and anti-mouse/rabbit F(ab’)_2, IgG HRP (Roche). Detection was performed using the BM Chemiluminescence Western Blotting kit (Roche Diagnostics). Nuclear extracts were isolated using the NE-PER reagent (Pierce) and the assessment of CREB-1, NF-κB p50, and NF-κB p65 binding activity was performed using the BD Mercury Transfactor Profiling kit (BD Biosciences).

**In vitro immunization assay**

In vitro immunization assays were performed as previously described (13). Briefly, DC and LC were incubated with or without HPV16-L1L2-E7 VLP/10^6 cells for 1 h at 37°C, as indicated. The cells were then mixed with autologous CD8+ T cells. Restimulations after 7 and 14 days were done with DC or LC treated as indicated. After 28 days effector cells were pooled and tested for IFN-γ production by ELISPOT as previously described (13).

**Results**

After HPV VLP encounter, LC down-regulate MAPK pathways

The signal transduction pathways initiated after Ag encounter in APC have a profound effect on the type of response the APC displays toward that Ag. APC may remain nonresponsive to the Ag encountered, or can stimulate a Th1 response, a Th2 response, or become inhibitory to the generation of an immune response (9, 10). It was previously found that human LC may be immunosuppressive after their encounter with HPV VLP, whereas human DC induced a potent CD8+ T cell response (13, 14). Those studies did not examine the signaling cascades initiated by the respective APC after HPV VLP encounter. Because the receptor for HPV has not been identified, we examined three signaling pathways frequently involved in the regulation of immune responses. The MAPK pathway, the NF-κB pathway, and the PI3-K pathway are all potential candidates downstream of a putative viral receptor.

The activation of MAPK pathways has been implicated in the response that APC display toward a variety of antigenic stimuli, therefore we examined the activation of MAPK pathways in DC and LC after encounter with HPV VLP. Within minutes after Ag encounter, many signal transduction molecules become phosphorylated and activated. Therefore we first determined the level of phosphorylated signaling molecules in DC or LC cellular extracts by Western blot analysis at 15 and 45 min poststimulation. After a 15-min incubation with HPV VLP, LC show a profound decrease in phosphorylated ERK1/2, MKK4, and ATF2 (Fig. 1a). LPS, a known inducer of MAPK signaling 24, up-regulated phosphorylated ATF2 in LC (Fig. 1, a and b), indicating that the LC used can respond to other stimuli. After a 45-min incubation, DC incubated with HPV VLP up-regulated phosphorylated ATF2 similar to LPS stimulation, whereas LC did not (Fig. 1b). The enhanced activation of signal transduction molecules in untreated LC as compared with DC can be attributed to the presence of TGF-β1-mediated signaling in LC (Fig. 1a). TGF-β1, which is required for LC differentiation (15, 16), induces activation of the pathways examined (17). However significant washes with PBS ensured that no free TGF-β1 was present during the incubations. We next determined the level of CREB-1 binding activity in nuclear extracts from treated DC and LC using a modified ELISA procedure (Fig. 1c). CREB-1 is a transcription factor activated downstream of MAPK signaling. We observed a significantly increased level of CREB-1 binding activity in extracts from DC treated with HPV VLP, similar to the positive control LPS, though not observed from HPV VLP-treated LC (Fig. 1c). When taken together, these data indicate that HPV VLP activate MAPK signaling cascades in DC, whereas MAPK pathways are suppressed in LC after HPV VLP stimulation.

The up-regulation of surface activation markers is a hallmark of APC activation and the activation of MAPK signaling has been implicated in their expression. Therefore, we examined the level of marker expression by DC and LC after treatment with SB203580, a potent MAPK inhibitor (18). DC treated with SB203580 did not up-regulate phosphorylated ATF2 after stimulation with LPS (data not shown), indicating a block in MAPK signaling. DC treated with HPV VLP up-regulate MHC class I, CD80, and CD86 compared with untreated DC as assessed by flow cytometry (Fig. 1d).

With the addition of the MAPK inhibitor, DC treated with HPV VLP did not significantly induce expression of any of the activation markers above untreated levels further indicating the importance of MAPK signaling in the activation of APC (Fig. 1d). As
expected, both LC incubated with the MAPK inhibitor and LC left untreated did not show a significantly increased level of marker up-regulation after incubation with HPV VLP (Fig. 1d).

After HPV VLP encounter, DC but not LC activate NF-κB

Another signaling molecule frequently found activated in APC after pathogen encounter is NF-κB (19). NF-κB is a dimer composed of two subunits, p50 and p65, normally held inactive in the cytoplasm by IκBα. Upon cellular activation, IκBα gets phosphorylated, ubiquinated, and subsequently degraded by the proteasome. This action allows the NF-κB dimer to translocate to the nucleus thereby initiating transcription from a variety of immune response genes (19). DC treated for 15 min with HPV VLP decreased the level of IκBα, whereas LC treated with HPV VLP did not (Fig. 2a). In nuclear extracts from DC treated for 45 min with HPV VLP, we found an increase in NF-κB p50 (Fig. 2b) and NF-κB p65 (Fig. 2c) binding activity, although not found with LC. This indicates that the NF-κB cascade is activated in DC and not in LC in response to HPV VLP. To explore whether NF-κB is partially responsible for the up-regulation of surface activation markers, we treated DC and LC with BAY11-7082, an inhibitor of IκBα processing, thereby inhibiting the activation and translocation of NF-κB (20). DC treated with BAY11-7082 and LPS show no decrease in IκBα degradation (data not shown), which indicates a block in NF-κB signaling. We treated DC and LC with or without BAY11-7082 and then with or without HPV VLP. We compared the level of cell surface MHC class I, CD80, and CD86 by flow cytometry after treatment with HPV VLP to the level expressed without addition of HPV VLP. The results indicate that incubation of DC with the NF-κB inhibitor limits the expression of the surface activation markers after treatment with HPV VLP (Fig. 2d), indicating a role for NF-κB in the HPV VLP-mediated activation of DC. Treatment of LC with BAY11-7082 resulted in no significant change in marker expression (Fig. 2d).

Activation of PI3-K by HPV VLP-stimulated LC inhibits the immune response

Class Iα PI3-K, which are activated by phosphorylation, are a subfamily of lipid kinases that have a diverse role in the regulation of many cellular responses (21). PI3-K activates protein kinase C (PKC), which is involved in various cellular responses, and Akt, shown to modulate both MAPK and NF-κB cascades (22). Previously it was shown that PI3-K activation in response to various stimuli negatively regulated the production of IL-12 by DC, and the inhibition of PI3-K resulted in enhanced immunity (23). After incubation with HPV VLP for 15 min, LC activated PI3-K,

![Figure 1](http://www.jimmunol.org/)
whereas DC displayed no detectable increase in phosphorylated PI3-K (Fig. 3a). Although Akt is downstream of PI3-K, Akt activation decreased in LC shown by a decrease in the amount of phosphorylated Akt (Fig. 3a). In DC treated for 15 min with HPV VLP, we observed an increase in phosphorylated Akt, indicating an increase in Akt activation. In control experiments, DC and LC incubated with HPV VLP that were heated for 10 min at 95°C to disrupt the VLPs structure resulted in levels of activated signaling molecules similar to the levels of untreated DC or LC (data not shown), indicating the importance of an intact viral structure for PI3-K signaling activation in LC. After a 45-min incubation with HPV VLP, we observed no increase in PI3-K activation in LC (Fig. 3b). However DC incubated with HPV VLP increased phosphorylated PI3-K and increased phosphorylated Akt similar to the positive control LPS (Fig. 3b). After 24-h incubation with HPV VLP, LC displayed an increased amount of phosphorylated Akt (Fig. 3c). In addition, DC and LC incubated with bovine papillomavirus VLP, a homologous type papillomavirus that activates both DC and LC, resulted in similar activation as was observed for DC plus HPV VLP (data not shown). These data, when taken together, indicate that LC activate PI3-K and suppress Akt early after encounter with HPV VLP, and then at later time points will activate Akt. DC activate PI3-K and Akt early and sustain this level of activation.

Because DC and LC incubated with HPV VLP differ in their activation of PI3-K, and because PI3-K activation has been implicated in the control of immune responses, we determined whether the activation of PI3-K by HPV VLP-treated LC plays a role in the lack of surface marker up-regulation. For these experiments we used LY294002, a potent specific inhibitor of PI3-K (24). We incubated DC and LC with or without LY294002, then with or without HPV VLP. DC treated with both the inhibitor and HPV VLP showed a higher fold increase in marker expression than DC treated with HPV VLP alone as assessed by flow cytometry (Fig. 3d). LC treated with only HPV VLP did not increase marker expression; however after treatment with LY294002 and HPV VLP, LC significantly increased marker expression (Fig. 3d). These data indicate that the activation of PI3-K suppresses the activation of signaling cascades required for surface marker up-regulation.

Because HPV VLP-treated LC significantly up-regulated activation markers only after incubation with the PI3-K inhibitor, we next sought to determine whether these LC could then initiate a CD8+ epitope-specific immune response. We performed an in vitro immunization procedure followed by IFN-γ ELISPOT analysis. The HPV VLP used in these experiments harbored HPV16-E7 protein, which contains a well-characterized human HLA-A*0201-restricted epitope (E786–93) recognized by human CD8+ T cells (25), fused to the L2 minor capsid protein. DC and LC generated from HLA-A*0201 positive donor PBL are capable of initiating an epitope-specific immune response to the E786–93 peptide after the in vitro immunization procedure (13). Previously it was shown that DC induce a potent response against E786–93 after incubation with HPV16-L1L2-E7 VLP, whereas LC require an additional activation stimulus such as CD40L (13). In the experiments presented in this study, DC and LC were treated with or without LY294002 and with or without HPV VLP. Then we incubated the cells with autologous naive CD8+ T cells and the cultures were restimulated twice with their respective treated DC or LC. Seven days after the last restimulation, the cells from each culture were collected and tested for a specific response to the HLA-A*0201-restricted HPV16-E7-derived peptide 86–93 by IFN-γ ELISPOT. As expected, DC loaded with HPV VLP initiated an epitope-specific response whereas loaded DC did not initiate a response (Fig. 4). However after treatment with the PI3-K inhibitor and HPV VLP, LC induced a response (Fig. 4), indicating that the lack of the LC ability to generate a response after treatment with HPV VLP alone could be overcome by the inhibition of PI3-K. Overall the data indicate that the activation of PI3-K by HPV VLP in LC suppresses the ability of LC to induce an immune response.

**In HPV VLP-stimulated LC PI3-K activates PKC, which activates PP2A**

To investigate the mechanism of suppression mediated by PI3-K in LC we used a set of potent specific molecular inhibitors and activators to target downstream members of the PI3-K pathway. We either left LC untreated or treated them with LY294002, PMA, or OA, which inhibits PI3-K (24), activates PKC (26), or inhibits PP2A (27), respectively. These LC were then incubated with or
without HPV VLP and the cellular extracts obtained were subjected to Western blot analysis. After treatment of LC with the PI3-K inhibitor LY294002, the level of phosphorylated Akt and ERK decreased compared with untreated LC (Fig. 5). These data indicate that, at the concentration used, LY294002 potently inhibits the action of PI3-K. PMA, a PKC activator, induced a decrease in phosphorylated Akt and an increase in phosphorylated ERK compared with untreated LC indicating that PKC activates ERK and also activates a phosphatase that inhibits Akt activation (Fig. 5). LC incubated with OA, a potent inhibitor of PP2A, resulted in enhancement of activation of each of the molecules examined (Fig. 5), indicating that PP2A controls multiple activation pathways in LC. Although OA also weakly inhibits protein phosphatase 1, a nuclear inhibitory subunit of protein phosphatase, NIPP-1 (28), did not result in enhancement of activation of any of these pathways (data not shown), indicating that PP2A controls multiple activation pathways in LC. Overall, PI3-K is the furthest molecule upstream in HPV VLP-mediated signaling of LC, which by all the inhibitors used shows similar levels of signaling molecule activation for untreated and HPV VLP treated LC except for PI3-K (Fig. 5). Taken together, these data indicate that PP2A is activated by HPV VLP in LC and that this activation is responsible for the lack of marker up-regulation and the inability of LC to induce an HPV-specific immune response.

**FIGURE 3.** Activation of PI3-K by HPV VLP-stimulated LC inhibits surface marker expression. Western blot analysis of elements of the PI3-K pathway in cellular lysates from DC and LC either untreated or treated with LPS or HPV VLP for 15 min (a), 45 min (b), or 24 h (c). The numbers below the blots represents the average density of the band relative to the band in untreated APC. d, Fold change in expression of MHC class I, CD80, or CD86 as determined by flow cytometry of DC and LC after stimulation with HPV VLP relative to untreated DC and LC, respectively. DC and LC were either untreated (−) or treated (+LY) with the PI3-K inhibitor LY294002.

**FIGURE 4.** Inhibition of PI3-K allows HPV VLP-stimulated LC to induce an HPV-specific response. ELISPOT analysis of chimeric HPV16-L1L2-E7 VLP loaded DC and LC treated with or without the PI3-K inhibitor against the known E7-derived HLA-A*0201-restricted CTL epitope (E7 peptide 86–93, 5'-TLGIVCPI-3') (25). * p < 0.005 determined by Student’s t test assuming one-tailed homoscedastic distribution.
In HPV VLP-stimulated LC PI3-K activates PKC, which activates PP2A. LC were either left untreated or treated with LY294002, PMA, or OA and subsequently incubated with or without HPV VLP for 15 min. Cellular extracts were subjected to Western blot analysis. The numbers below the blots represents the average density of the band relative to the band in untreated APC.

**FIGURE 5.** In HPV VLP-stimulated LC PI3-K activates PKC, which activates PP2A. LC were either left untreated or treated with LY294002, PMA, or OA and subsequently incubated with or without HPV VLP for 15 min. Cellular extracts were subjected to Western blot analysis. The numbers below the blots represents the average density of the band relative to the band in untreated APC.

**FIGURE 6.** Model for signaling initiated in LC after HPV VLP encounter. After HPV VLP encounter, PI3-K activates PKC, which activates PP2A. PP2A inactivates Akt and the MAPK, ERK, and NF-κB pathways resulting in no increase in transcription from immune response genes. PKC could also directly bind Akt thereby inhibiting Akt activation. Inhibition of PI3-K would relieve the suppressive mechanisms in LC, thereby allowing transcription from immune response genes.

Discussion

The high prevalence of HPV-associated diseases in the world, of which cervical cancer is the third most common cancer among women (2), indicates that HPV must have evolved mechanisms to evade host immune recognition resulting in increased viral transmission and increased incidence of disease. In this study we present data targeting LC, the APC found at the sites of primary HPV infection, as a mechanism used by HPV to evade host immunity. After HPV VLP encounter, LC activate the PI3-K pathway, which in turn down-regulates Akt and ERK activation. This down-regulation is mediated by PP2A, which has previously been shown to down-regulate Akt, PKC, MAPK, ERK, and the 1κB kinases (29), thereby regulating multiple signaling cascades involved in the control of immune responses (Fig. 6). PKC also has been shown to inhibit Akt activation independent of PP2A (30). However, we show that specific inhibition of PP2A resulted in restoration of Akt activation. Akt has a biphasic role in the regulation of multiple signaling pathways. Early, Akt activates NF-κB (31, 32), whereas later, Akt down-regulates MAPK pathways and inhibits 1κB kinase, thereby inhibiting NF-κB (33). We found that HPV VLP stimulation of LC results in early suppression of Akt by 15 min, and later activation of Akt at 45 min to 24 h, indicating that HPV VLP inhibit all phases of a possible response.

Previous data have shown that HPV VLP are endocytosed, processed, and presented in the context of MHC class I by LC; however, they do so in the absence of costimulation (13, 14). Therefore whether LC presenting HPV peptides on their surface come into contact with HPV-specific T cells, the T cells will not respond and may even become suppressed. After inhibition of PI3-K and treatment with HPV VLP, LC up-regulate surface activation markers and are capable of initiating a potent HPV VLP-specific immune response. This result indicates that the activation of PI3-K and the subsequent activation of PP2A by HPV VLP in LC defines a mechanism of immune escape used by HPV. The targeting of LC by HPV is a function of the intact viral protein capsid, as we show that HPV VLP with a disrupted structure and bovine papillomavirus VLP, which have a different protein makeup, do not induce PI3-K activation.

As a vaccine, HPV VLP induce a potent anti-HPV immune response. This is a result of the HPV VLP being injected to target DC. After encounter with HPV VLP, DC initiate the MAPK, NF-κB, and PI3-K signaling cascades. The activation of MAPK and NF-κB cascades in DC results in the up-regulation of surface activation markers and affords these cells the ability to initiate an immune response. PI3-K, also activated by DC in response to HPV VLP, but not as profound as in LC, partially negatively regulates marker expression, although not enough to hinder the DC ability to induce an immune response. Overall the data indicate that DC and LC respond differently to HPV VLP and that the activation characteristics observed are specific to HPV viral particles.

The data also indicate that an inhibitor of ERK-, MAPK-, and NF-κB-mediated expression exists in unstimulated LC (Fig. 6). This observation is due to the fact that untreated LC display an increased amount of these signaling molecules activated even though they do not display an enhanced level of surface activation markers. TGF-β1, which is required for LC differentiation (15, 16), was previously shown to activate the PI3-K pathway (17) and TGF-β1 may drive the action of the inhibitor. In accordance with this observation DC, which do not require TGF-β1, do not show a similar level of activated signaling molecules as LC, although both DC and LC have similar baseline levels of surface activation markers. Also treatment of LC with the PI3-K inhibitor, which would relieve the action of the inhibitor, allows the LC to respond to HPV VLP stimulation resulting in marker expression.

Previously it was shown that HPV-associated lesions displayed an increased PI3-K activity and a decreased activation of Akt (34). These authors concluded that the results obtained were due to the increased expression of the phosphatase and tensin homolog deleted on chromosome 10 (PTEN), a negative regulator of Akt activation. We did not observe any change in PTEN levels in our DC and LC extracts (data not shown) and it is clear from the data obtained that PP2A controls the inactivation of Akt in LC. Therefore another stimulus may be causing the increased PTEN expression after lesion formation. When taken together, this finding indicates that the activation of PI3-K is involved in many steps in
lesion development and the escape of immunity. Also this finding suggests that in addition to a newly identified immune escape mechanism used by HPV, PI3-K may serve as an effective clinical target for inhibition to enhance HPV immunity.

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Disclosures
The authors have no financial conflict of interest.

References


