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Control of immune escaped human papilloma virus is regained after therapeutic vaccination

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High-risk human papillomaviruses infect the basal cells of human epithelia. There it deploys several mechanisms to suppress pathogen receptor recognition signalling, impeding the immune system to control viral infection. Furthermore, infected cells become more resistant to type I and II interferon, tumour necrosis factor-α and CD40 activation, via interference with downstream programs halting viral replication or regulating the proliferation and cell death. Consequently, some infected individuals fail to raise early protein-specific T-cell responses that are strong enough to protect against virus-induced premalignant disease and ultimately cancer. Therapeutic vaccines triggering a strong T-cell response against the early proteins can successfully be used to treat patients at the premalignant stage but combinations of different treatment modalities are required for cancer therapy.

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Introduction

Progressive infections show split immunity to HPV late and early proteins

About 80% of sexually active individuals become infected with a high-risk HPV type (hrHPV). While most hrHPV infections (90%) are controlled within two years [1], viral persistence may lead to malignancies. The hrHPV are responsible for ~5% of all human cancers. Of the 14 different hrHPV types detected in cervical carcinoma, HPV16 and 18 are the most prevalent. HPV16 is the dominant type in all other HPV-induced cancers [2,3].

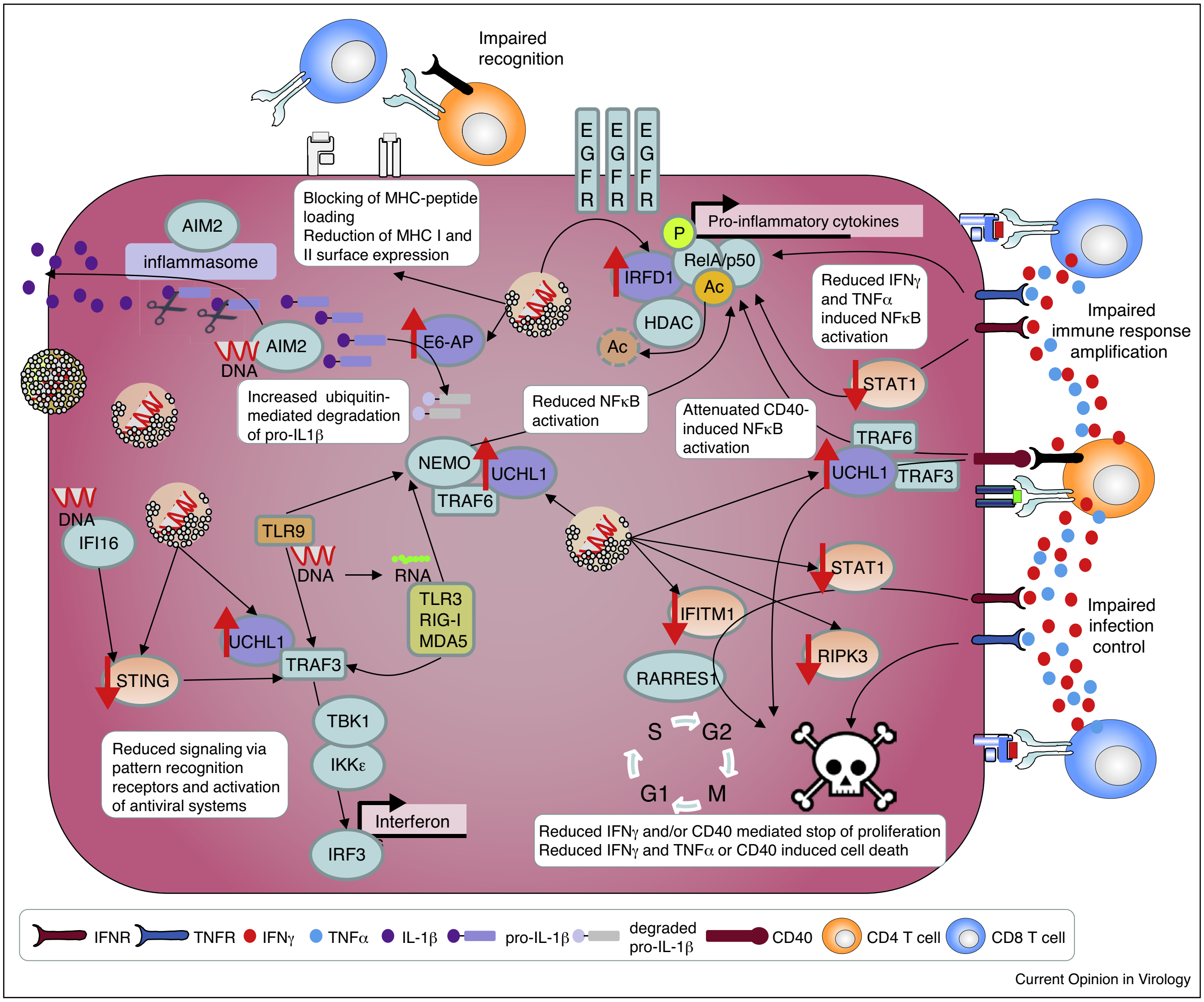
HPV exclusively infect keratinocytes (KCs) in the basal layer of the epidermis and mucosal epithelia, through micro-wounds and abrasions. In the large majority of exposed but healthy individuals strong type 1 (IFNγ, TNFα, IL-2 producing) T-cell responses to the structural protein L1 as well as the early proteins E2, E6 and E7 are detected [4–7]. Stimulation of the L1-specific immune response most likely occurs via the uptake of virions, produced during the productive phase of the infection, by the Langerhans cells that reside in the epidermis. T-cell responses to L1 are detected in healthy individuals and in patients with premalignant lesions or cancer [7]. While they reflect a productive infection, they do not contribute to the control of viral infection as L1 is not expressed in the first few layers of the proliferating infected basal cells. In these layers, however, the early proteins E2, E6 and E7 are produced and immunity may be induced if these proteins are taken up by professional antigen presenting cells. However, type 1 T-cell responses to the early proteins are weak at best in patients with persistent infections.

The HPV infected skin expresses the cytosolic DNA sensors STING, AIM2 and IFI16. HPV DNA can trigger the latter two resulting in the secretion of IL-1β and IL-18 [8]. These cytokines mediate local and systemic immune responses to infection [9] and might be critical for early immune control of virus replication [10–12]. Hence, there is a period in which an HPV infection may trigger a protective T-cell response, dominated by CD4+ T-cells [5,6,12,13,14•], but if this response is too weak or too late HPV may deploy several mechanisms to suppress the pathogen recognition receptor pathways [15–19,20••,21–23]. Importantly, as HPV infection does not cause viremia or cell lysis, either intact immune signalling or minor trauma to the lesion [24] is crucial to induce protective immunity.

Mechanisms used by HPV to prevent immune control

Basal KCs express several pattern-recognition receptors (PRR) that can recognize viral DNA or RNA (Figure 1). PRR ligation results in the production of type I interferon and pro-inflammatory cytokine production through signaling via interferon regulatory factor (IRF) and nuclear factor of kappa-light-chain-enhancer of activated B cells (NFκB) activating pathways. Several genome-wide transcription studies reported that hrHPV types have found means to suppress PRR- and type I IFN-induced signaling pathways [22]. Recently it was found that the cells in hrHPV-positive low-grade lesions display higher levels of

Figure 1



High-risk human papillomavirus deploys countermeasures to prevent immune control.
High-risk HPV can infect basal keratinocytes. The virus can be recognized by the pattern recognition receptors for viral DNA: IFI16, AIM2, TLR9 and for viral RNA: TLR-3, RIG-I, MDA-5. Most of these will activate interferon production via TRAF3-TBK1-IKKε-IRF3 interactions but this is prevented by downregulation of STING and the upregulation of UCHL1, which inactivates TRAF3 via deubiquitination. UCHL1 also suppresses TLR9 and TLR3/RIG-I/MDA5-mediated activation of NFκB via interaction with TRAF6 and degradation of NEMO. While viral DNA may activate the formation of the AIM2 inflammasome, required to cleave pro-IL1β into the potent immune activating cytokine IL-1β, the upregulation of E6-AP results in the ubiquitination of pro-IL1β targeting it for proteasomal degradation. Activated CD4+ type 1 T cells express CD40L and produce IFNγ and TNFα. Activation of CD40 and the IFNγ receptor (IFNR) result in proliferative arrest of cells, but this is impaired by the downregulation of STAT1 and IFITM1 (downstream of IFNR) and deactivation of TRAF3 (downstream of CD40) by UCHL1, with as result less upregulation of the antiproliferative gene RARRES1. RIPK3 is one of the key components in necroptosis, which is down-regulated by hrHPV, resulting in reduced IFNγ and TNFα induced necroptosis. High-risk HPV induces the overexpression of epidermal growth factor receptors (EGFR) and this increases the expression of IFRD1. IFRD1 mediates RelA K310 deacetylation thereby attenuating the transcriptional activity of NFκB. The resistance will be similar to CD8+ T-cell produced IFNγ and TNFα. Black arrows indicate the normal reactivity in the cell after stimulation. The purple proteins are upregulated and orange proteins are downregulated as a result of hrHPV infection.

E2 than normal hrHPV-infected cells, and this coincided with downregulation of STING [20••]. Furthermore, hrHPV upregulated UCHL1, a deubiquitinase which was shown to inactivate TRAF3 and mediates the degradation of NEMO [15] and it may inhibit TLR9 expression [25]. Notably, prednisolone- and hydroxychloroquine-mediated downregulation of TLR7 and TLR9, respectively, is associated with HPV infections [26]. As a consequence, persistently hrHPV-infected cells will be less equipped to attract and activate the adaptive immune response via the production of interferons and cytokines (Figure 1). Especially, the secretion of the potent immune activating cytokine IL-1β is suppressed by hrHPV by targeting pro-IL-1β for destruction [27].

However, even when the immune system manages to mount a type 1 T-cell response it will be difficult for these T cells to control a persistent infection as hrHPV adapts the infected cells to become less sensitive to immune control mechanisms (Figure 1). The virus interferes with T-cell recognition via the reduction of MHC class I and II expression but also by affecting the downstream signalling pathways of CD40, and the TNFα and IFNγ receptors which normally will mitigate the infection by arresting cell proliferation and inducing cell death, but will also lead to amplification of the local immune response via the direct (CD40, TNFα) and indirect (IFNγ) activation of NFκB (Figure 1). Persistently hrHPV infected cells display lower levels of STAT1 but this does not completely impair signalling [28,29••]. Therefore, hrHPV also downregulate the interferon-induced transmembrane protein 1 (IFITM1) thereby preventing the upregulation of the antiproliferative gene RARRES1 [29••]. A similar suppression of RARRES upregulation is noted after CD40 ligation [30]. In addition, hrHPV evades TNFα-induced cell death of infected cells by the downregulation of RIPK3, a crucial regulator of necroptosis [29••]. Local amplification of immunity by the secretion of cytokines and the attraction of immune cells is dampened by hrHPV through an increased expression of interferon-related developmental regulator 1 (IFRD1), which attenuates the transcriptional activity of NFκB via deacetylation of RelA [31••] as well as by interfering with downstream signalling of CD40, probably via the interaction of UCHL1 and TRAF6 [15,30]. Finally, there is evidence that hrHPV-infected cells create a local immune suppressive microenvironment by altering the phenotype and function of local antigen dendritic cells [32] and the attraction of mast cells [33].

A strong vaccine-induced type 1 T-cell response regains control of HPV-induced diseases

Therapeutic vaccines aim to stimulate strong type 1 helper T-cell and cytotoxic T-cell responses (Th1/CTL) to attack infected cells. They come in many flavours [34] and are also developed to treat HPV-induced diseases [35].

Clinical success has been obtained in women either with infected cells or with hrHPV-induced high-grade lesions. GTL001 in combination with the TLR7 agonist imiquimod topically applied to the vaccine site as adjuvant, stimulated E7-reactivity and a post-hoc analysis suggested increased and sustained clearance of HPV, albeit that the group size was small [36].

Four different types of vaccines were tested for their capacity to treat hrHPV-associated high-grade cervical lesions (CIN2-3). The DNA vaccine VGX-3100 was shown to induce strong E6/E7-specific Th1/CTL responses [37••] and was subsequently tested in a large randomized placebo-controlled trial [38••]. The spontaneous clearance rate of CIN2-3 was 30% and this was increased to 50% by vaccination. Post-hoc analyses revealed a relation between a clinical response and the strength of the vaccine-induced immune response [38••]. Also the DNA vaccine GX-188E induced E6/E7-specific Th1/CTL responses that resulted in viral control and lesion regression in 7 out of 9 patients [39] while another (pnGVL4a-CRT/E7 DNA) failed to induce strong Th1/CTL reactivity or clinical reactivity exceeding the spontaneous clearance rate [40]. GLBL101c, an orally administered bacterial vector vaccine expressing HPV16 E7 protein [41] did not lead to overt systemic immunity but HPV-specific T-cells were detected in the cervix. A downgrade of disease stage was found in 5 of 13 patients [41], just above the spontaneous clearance rate. Similarly, PepCan, an HPV16 E6 peptide-based vaccine with Candida skin test reagents as adjuvants induced T-cell reactivity in $<50\%$ of the subjects and there was no relation between immunity and lesion regression or an increase in clearance rate [42,43]. The spontaneous clearance of HPV16-induced high-grade lesions of the vulva is less than 1.5% and treatment with the synthetic long peptide vaccine ISA101 considerably increased this percentage to more than 50% as shown in two subsequent medium-sized trials [44,45••]. Clinical reactivity was strongly related to the strength of the vaccine-induced Th1/CTL response as found during the post-hoc analyses of the first trial [44,46] and confirmed as pre-defined marker in the second trial [45••].

The general observation from these trials is that if a strong Th1/CTL response is evoked one has the best chance for a clinical response. This fits with studies showing that hrHPV increases the resistance of infected cells to the effects of type 1 cytokine mediated signals but does not make them insensitive [15,23–25,29••,31••,42]. In addition, it should be appreciated that the viral gene expression changes during the progression of disease and this may impact on the immune evasive strategies deployed [22]. For example, STING expression is regained in progressive lesions, consistent with the loss of E2 protein expression [23].

Currently 20 different ongoing trials focus on the treatment of premalignant or cancerous lesions (Table 1). Bearing in mind that local immune suppression hampers the efficacy of therapeutic vaccines [34] there are a couple of trials attracting the attention. Two trials try to circumvent general immune suppression by vaccinating patients during cancer surgery or after successful

Table 1

Current therapeutic vaccine trials

Vaccine	Goal	Disease stage	Status	NCT#
PDS0101	Safety, tolerability and pharmacodynamics of Versamune® + Peptides from HPV16 E6&E7	Women with infection or CIN1	Recruiting	02065973
VB10.16	Safety and immunogenicity of an HPV16 E6&E7 DNA vaccine targeted to antigen presenting cells	CIN2-3	Not recruiting	02529930
pnGVL4a-CRT/E7 DNA & topical imiquimod	Safety and efficacy of intralesional administration and Imiquimod treatment of lesion	CIN2-3	Recruiting	00988559
TA-HPV + Sig/E7/ HSP70 DNA & topical imiquimod	Safety and efficacy of vaccination with Imiquimod treatment of lesion	CIN3	Recruiting	00788164
PepCan	Efficacy and safety of HPV16 E6 peptides & Candin adjuvant	CIN2-3	Recruiting	02481414
GX-188E	Determine recurrence of CIN and evaluation of long-term safety	CIN3	Recruiting	02411019
ISA101 & IFNα as immune modulator	Safety, immunogenicity and efficacy of different intradermal doses HPV16 E6 and E7 synthetic long peptides with or without pegylated IFNα	AIN2-3	Recruiting	01923116
TA-HPV	Immunogenicity and impact on DFS when injected at time of surgery	Early cervical cancer	Completed	00002916
GM-CSF treated PBMC with E6/E7 peptides	Immunogenicity and efficacy of vaccination	Advanced or recurrent cancer	Completed	00019110
ADXS11	Immunogenicity and impact on 1 year survival of live-attenuated Listeria monocytogenes E6&E7 vaccine	Advanced or recurrent cancer	Suspended	01266460
BVAC-C	Safety and immunogenicity of recombinant HPV16/18 E6/E7 expressing Adenovirus-infected B-cells and monocytes	Advanced or recurrent cervical cancer	Recruiting	02866006
INO-3112	Safety and immunogenicity of VGX-3100 plus DNA-based immune activator encoded for IL-12	Advanced or recurrent cancer	Not recruiting	02172911
INO-3112	Safety and immunogenicity when delivered by electroporation	Head and neck cancer	Not recruiting	02163057
ADXS11	Immunogenicity and toxicity of live-attenuated Listeria monocytogenes E6&E7 vaccine injected before surgery	Oropharyngeal cancer	Recruiting	02002182
ISA201	Biological activity of two HPV16 E6 specific peptides coupled to a Toll-like receptor ligand	Non-metastatic oropharyngeal cancer	Recruiting	02821494
P16_37-63 peptide in Montanide ISA51 & chemotherapy	Immunogenicity and safety of p16 peptide vaccination during cisplatin chemotherapy	HPV- and p16INK4a-positive cancer	Not recruiting	02526316
ISA101/101b in Montanide ISA51 & chemotherapy	Safety and immunogenicity of different doses HPV16 E6&E7 long peptides with or without pegylated IFNα as combination therapy with carboplatin and paclitaxel	Advanced or recurrent HPV16-induced cancer	Recruiting	02128126
DPX-E7 & chemotherapy	Safety and efficacy of a single HLA-A2-restricted HPV16 E7 epitope with metronomic cyclophosphamide	HPV-induced cancers	Not yet recruiting	02865135
ISA101 in Montanide ISA51 & Nivolumab	Phase 2 efficacy study of ISA101 with PD-1 checkpoint inhibition	HPV16-positive incurable cancers	Recruiting	02426892
TA-CIN & GPI-0100 adjuvant	Safety and feasibility of HPV16 L2-E6-E7 fusion protein with triterpene glycoside adjuvant	History of HPV16-positive cervical cancer	Not yet open	02405221

standard treatment, aiming to prevent recurrences (NCT00002916; NCT02405221). In three trials vaccination is combined with chemotherapeutics that may alleviate immune suppression mediated by regulatory T cells (NCT02865135) or myeloid cells (NCT 02526316; NCT02128126) [47•,48•]. Last but not least, activated T cells may express PD-1, which after engagement with PD-L1 on tumor cells or myeloid cells, suppresses their effector function. In one trial this is prevented by combining vaccination with the PD-1 blocking antibody nivolumab (NCT02426892).

Conclusion

The high incidence of HPV infections, the quick clearance of infections in spite of HPV's stealthy behaviour, and the detection of early protein-specific T cells in most healthy subjects while seroconversion is low, indicates that in general pathogen recognition of hrHPV occurs after which a protective T-cell response is launched. The production of IL-1β may be crucial for the activation of a strong T-cell response during hrHPV infection. IL-1β is important for the acute phase response and it also enhances the expansion, differentiation and tissue localization of CD4+ and CD8+ T-cell responses [49,50]. However, polymorphisms in the IL-1 gene [12,51] and active downregulation of a network of IL-1β interconnected genes by hrHPV [16] as well as inhibition of IL-1β secretion at higher stages of disease [27] may stifle the development of protective type 1 T-cell responses in a minority of cases, with weak T-cell reactivity as result. In addition, hrHPV lowers the sensitivity of infected cells to key type 1 cytokines which otherwise will help infected cells to control the virus and it creates a local suppressive environment. This raises the bar for the type 1 T-cell responses to gain control of infection but therapeutic vaccines can stimulate type 1 HPV-specific T cell responses with a magnitude that readily exceeds the weak responses in patients and this is associated with regained control of hrHPV infection. In time, however, additional layers of immune suppression develop within the hrHPV-induced lesion necessitating combinations of vaccines with other treatment modalities to alleviate these suppressive mechanisms.

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- •• of outstanding interest

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