SPONTANEOUSLY INDUCED CELLULAR IMMUNITY TO HPV-16/18 AND THE THERAPEUTIC VACCINATION OF PATIENTS WITH HPV-16/18 (PRE-)MALIGNANT LESIONS

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Cervical carcinomas arise as result of an uncontrolled persistent infection with a high-risk type of human papillomavirus, in particular types 16 (HPV-16) and 18 (HPV-18) that account for approximately two-third of these cancers. The HPV E6 and E7 proteins play a pivotal role in carcinogenesis and are expressed in both pre-malignant and advanced cervical lesions. Because HPV proteins are foreign to the body, one would expect the immune system to mount a response against these antigens when expressed in the cervical epithelium. Indeed, circulating HPV-16 E6, E7 and E2-specific Th1- and Th2-type CD4+ T-cells, able to migrate into the skin upon antigenic challenge, were frequently detected in healthy individuals (1–5), showing that successful defense against HPV-16 infection is commonly associated with the installment of a systemic effector T-cell response against these viral antigens. In contrast, the development of high-risk HPV-positive cervical cancer is associated with a HPV-specific T-cell response that fails at least at three different levels. In about half of the patients, PBMC lack the capacity to mount a detectable proliferative response against HPV-16 E6, E7 and E2, whereas the other half displayed antigen-specific proliferative responses exhibiting a non-inflammatory cytokine profile (6). Analysis of the local immune response revealed that in many cases HPV-specific effector T-cells failed to home to the tumors or to infiltrate the cancer cell nests (7, 8). Furthermore, we recently demonstrated that HPV E6- and E7-specific CD4+ regulatory T-cells can be isolated from lymph node biopsies of cervical cancer patients and, moreover, that such T-cells can infiltrate tumors, suggesting that anti-tumor immunity in cervical cancer patients is suppressed at both the induction and effector level (9). Notably, the viral antigens concerned are the prime components of all therapeutic vaccines against cervical cancer that are currently under development (10, 11). Although such vaccines are designed to enhance CD4+ and CD8+ T-cell effector immunity against the E6 and E7 oncoproteins of HPV-16 and/or -18, the presence of pre-existing E6 and E7-specific CD4+ regulatory T-cells in lymph nodes and tumors from cervical cancer patients brings forward the possibility that vaccination might also – or instead – result in activation and expansion of this regulatory T-cell subset. Indeed we observed that in addition to the expansion of HPV-16-specific CD4+ effector T-cells (12–13) also a population of HPV-16-specific CD4+Foxp3 regulatory T-cells expanded after vaccination (13). Our data indicate that strategies to eliminate or disarm regulatory T-cells prior to vaccination, which are now widely considered in the context of modalities that aim at inducing effective immune responses against tumor-associated auto-antigens, should also be considered for immunotherapeutic strategies against cancers with viral etiology.

REFERENCES

Summary

Various high-risk HPV (HR HPVs) DNA assays have been developed that allow detection of a broad spectrum of HR HPVs. Two of these assays [Hybrid Capture 2 (hc2) and GP5+/6+-PCR] have shown in large clinical trials a superior clinical sensitivity for cervical (pre)cancer compared to cytology and an optimal balance between clinical sensitivity and specificity. Comparative studies showed that an increased sensitivity for HR HPV relative to GP5+/6+-PCR and/or hc2 results in a dramatic decrease in clinical specificity, whereas on the other hand a decreased sensitivity for virus leads to a decrease in specificity for (pre)cancer. These data argue for guidelines on HR HPV test requirements for cervical screening purposes.

Key words: human papillomavirus, cervical cancer, high-grade CIN, HPV DNA testing, clinical performance

The fact that high-risk human papillomavirus (HR HPV) infection is a necessary cause of cervical cancer offers possibilities to implement HR HPV testing in cervical cancer screening to improve the efficacy of the screening program. Many test systems have been developed that can detect the broad spectrum of HR HPV types in one assay. An extensive overview of available HPV detection methods has recently been described (1). Most of these HR HPV assays comprise DNA detection methods based on either target amplification utilizing PCR or signal amplification.

As a result of the heterogeneity between HR HPV types, the majority of DNA PCR methods make use of consensus primers that target the same conserved region within the viral genome, though also multiplex systems targeting different regions for different types in one reaction have been described. The most commonly applied consensus PCR assays include GP5+/6+-PCR (a modified version of GP5/6), PGMY09/11 (a modified version of MY09/11), and SPF10 (1). Detection of a PCR product can easily be performed by enzyme-immuno-assays (EIA) that use a cocktail of type-specific or a mix of universal probes. Genotyping on the generated PCR products is mostly carried out by reverse hybridization formats using type-specific oligonucleotide probes immobilized to filters/strips (like reverse line blot (RLB), line probe assay (LiPA), linear array), micro-arrays (micro-chip based oligonucleotide arrays), or microsphere beads (bead-based multiplex HPV genotyping). According to our experience, the various reverse hybridization on consensus GP5+/6+-PCR products perform equally well and reveal highly concordant typing results. Hence, the clinical value of these typing assays largely depends on the clinical performance of the PCR system generating the products that are used for typing.

Other HPV DNA detection assays are based on signal amplification and either have a liquid-phase or an in situ hybridization (ISH) format. The commercially available and FDA (USA) approved hybrid capture 2 (hc2), QiaGen, Gaithersburg, MD, USA) is an example of a liquid-phase format and detects 13 genital HR HPV types using a mixture of full-length RNA probes. Unlike PCR formats, the signal amplification formats do not enable HPV genotyping in one assay run.

With respect to all above mentioned assays, it should be realized that for screening and clinical practice purposes the detection of HR HPV is not inherently useful unless it is found in the context of cervical precancer (high-grade cervical intraepithelial neoplasia – CIN) or cancer. With regard to this aspect, there is sufficient evidence that the viral load (ie. the amount of viral DNA in a sample) is an important variable since very low viral loads reflect clinically irrelevant, mostly transient, HR HPV infections. As such, detection of such low copy numbers of viral DNA will have a negative impact on the clinical specificity for detection of high-grade CIN and cervical cancer. In fact, for screening purposes, HR HPV DNA tests should reach an optimal balance between clinical sensitivity and specificity. Current clinically