Review

The status of HPV16-specific T-cell reactivity in health and disease as a guide to HPV vaccine development

Sjoerd H. van der Burg *, Annemieke de Jong, Marij J.P. Welters, Rienk Offringa, Cornelis J.M. Melief

Tumor Immunology Group, Department of Immunohematology and Blood Transfusion, University Medical Center, Albinusdreef 2, 2333 ZA Leiden, The Netherlands

Abstract

Human papilloma viruses (HPV) are among the most common sexually transmitted pathogens in young adults. In the majority of individuals, anti-viral immunity is capable of suppressing viral infection but in a minority of patients viral infection is not cleared in time to prevent the development of malignancies. In these cases, HPV16-specific immunity may develop too late, is not strong enough, and/or is possibly of the wrong type. The influence of pre-existing immunity on the efficacy of vaccines is largely unknown. Nor has it been studied what the effect is of vaccines on the various types of pre-existing HPV-specific T-cell immunity. Animal models showing that vaccines are able to protect against a subsequent tumor challenge and even to treat transplantable tumors, are not qualified to address this point because tumor development is not preceded by persistent viral infection. Therefore, the comparison between fully characterized pre-existing HPV-specific immunity in patients and healthy subjects is a prerequisite for the full appreciation of vaccine-efficacy as well as for further development of next-generation vaccines.

Keywords: HPV; T-cell immunity; Vaccine

1. Introduction: vaccine development in relation to pre-existing HPV16-induced immunity

The oncogenic types of human papilloma virus (HPV) cause several types of human cancer, in particular cervical carcinoma (zur Hausen, 1996). HPV16 DNA is detected in > 50% of all cervical tumors and the expression of HPV16 derived E6 and E7 onco-proteins is essential for maintaining the transformed state. The manifestation of these viral antigens lies at the basis of the development of therapeutic vaccines for the treatment of HPV16-induced lesions. Candidate vaccines, targeting these viral antigens, were able to induce effective HPV16-specific T-cell responses that protected mice against a subsequent HPV16+
tumor-cell challenge (De Bruijn et al., 1998; Feltkamp et al., 1993; van der Burg et al., 2001a). Some of these vaccines even possessed the capacity to eradicate tumors in mice (Chu et al., 2000; Gunn et al., 2001; Lamikanra et al., 2001; Velders et al., 2001; Zwaveling et al., 2002). This demonstrates that prophylactic as well as therapeutic vaccination against HPV16-positive tumors, is possible.

In human beings, however, tumors arise from epithelia that are persistently infected with HPV16, and virus-specific T-cell responses may develop after infection has been established. Studies addressing the L1-specific antibody response, demonstrated a delay between time of infection and onset of immunity (Dillner, 1999). Despite this delay, anti-viral immunity is aroused in the majority of infected subjects and is capable of suppressing viral infection. However, in a minority of cases viral infection is not cleared in time to prevent the development of malignancies. HPV16-specific immunity may develop too late, may not be strong enough, and/or may even be of the wrong type. Although animal models are truly helpful, they cannot be employed to address this point, because tumor development is not preceded by a period of persistent viral infection. Furthermore, transplantable tumors grow outside the context in which tumors normally arise. In addition, these tumors grow fast and simply outpace the immune system.

Hence, candidate vaccines are likely to be used in humans against different backgrounds of HPV-specific immunity. How these different types of pre-existing HPV-specific T-cell immunity influences the effectiveness of vaccines is largely unknown. Nor has it been studied what the effect is of vaccines on the various types of HPV-specific T-cell immunity. In our opinion, the efficacy of vaccines can only be fully appreciated when it is set off against the HPV-specific immune status of the individuals receiving these vaccines. This requires comparison between fully characterized pre-existing HPV-specific T-cell immunity in patients with a wide variety of HPV-related disorders of the uterine cervix, and the HPV-specific immune status in healthy individuals.

2. A role for T-cell immunity in the protection against HPV-induced progressive lesions

Genital infection of both men and women with oncogenic HPV types is common (Burk et al., 1996; Karlsson et al., 1995; Koutsky, 1997) but only a minor fraction of infected subjects develop progressing epithelial lesions or cancer (Duggan et al., 1998; Evander et al., 1995; Ho et al., 1998). Most pre-malignant lesions regress spontaneously and this is likely mediated by cellular immune responses, as suggested by the fact that regressing genital warts are infiltrated with increased numbers of activated CD4+ T-cells and CD8+ T-cells (Coleman et al., 1994). The role of T-cell immunity in controlling infection by various HPV types is further indicated by the increased incidence of HPV infections, HPV-associated warts, cervical intraepithelial neoplasia (CIN) lesions and cervical carcinoma in immunocompromised subjects such as transplant patients or HIV positive subjects (Benton et al., 1992; Palefsky et al., 1999; Serraino et al., 1999). This notion is further sustained by the observations that papillomavirus-specific T-cell immunity can protect against malignant transformation of papillomas in rabbits (Selvakumar et al., 1995a,b). Finally, T-cell immunity can prevent the outgrowth of HPV16+ tumors (De Bruijn et al., 1998; Feltkamp et al., 1993; Greenstone et al., 1998) and even eradicate established HPV16+ transformed tumors in mouse models (Feltkamp et al., 1995; Gunn et al., 2001; Lamikanra et al., 2001; Velders et al., 2001; Zwaveling et al., 2002). In conclusion, cellular immunity against HPV most likely plays a role in the protection against HPV-induced disease.

3. HPV16-induced T-cell immunity in patients

3.1. Immunomonitoring, the ‘old way’

Screening of current literature on HPV-specific T-cell immunity reveals a plethora of animal studies and vaccination strategies. In contrast, only a few studies address HPV-specific T-cell immunity in man. In general, immune reactivity against the HPV16 E6 and E7 oncoproteins was
analyzed, because these two proteins are uniformly expressed in all HPV16-induced cancers. Occasionally, L1 or E2-specific reactivity was examined. Stimulation of PBMC with recombinant proteins or overlapping sets of synthetic peptides demonstrated E6 and/or E7 T-helper (Th) reactivity in patients with progressive disease or cervical cancer. Depending on the study < 30% of patients (Luxton et al., 1996; Tsukui et al., 1996), half of the patient group (Kadish et al., 1994) or an ever higher percentage of patients (de Gruijl et al., 1998, 1996; Kadish et al., 1997) responded against HPV16 E7. In studies that measured HPV16 E6- and E7-specific T-cell reactivity in parallel, the response rate to E6 was similar to that of E7 (Kadish et al., 1997; Tsukui et al., 1996). L1-reactivity was found in all patients with current HPV16 infection and in most patients with CIN III (de Gruijl et al., 1999; Shepherd et al., 1996).

To assess HPV-specific cytotoxic T-lymphocyte (CTL) activity, HPV16+ patient derived PBMC have been stimulated with recombinant protein, defined minimal peptide–epitopes or with recombinant adenovirus expressing HPV16 E6 and E7 infected PHA blasts for 7–21 days. In some studies, CTL reactivity against both HPV16 E6 and E7 was predominantly found in patients that cleared infection (Nakagawa et al., 1997, 2000). In other studies, especially patients with persistent infections or progressive disease displayed CTL reactivity (Bontkes et al., 2000; Evans et al., 1997; Nimako et al., 1997; Ressing et al., 1996).

The conspicuous difference in the number and type of responders between these studies is most likely explained by the methods that were used to analyze the data. In our opinion, low-stringent criteria to define HPV-specific T-cell reactivity were used. For example, antigen-specific IL-2 production that exceeded twice the background production of non-stimulated cells, classified patients as responders (Bontkes et al., 1999; de Gruijl et al., 1998, 1996, 1999; Luxton et al., 1996; Tsukui et al., 1996). Furthermore, HPV-specific CTL activity, measured by chromium-release assays after one or more in vitro stimulations, was defined to be present when at 2 or more effector to target ratio’s a difference of 10% lysis with the control was observed (Bontkes et al., 2000; Nakagawa et al., 1997, 2000; Nimako et al., 1997). Obviously, the proportion of actual responders is strongly influenced by the number of T-cell cultures that display borderline reactivity in these assays. Are these responses truly reflecting the in vivo situation? In our view, this still needs to be determined because long in vitro culture periods may activate naïve T-cells capable of responding to the offered antigen (ten Bosch et al., 1996; van der Burg et al., 1999; Ressing et al., 1995).

From these data, a general picture of HPV16-specific immunity in human beings emerges. It is clear that at one point during HPV16-infection, Th-cells and CTL reactive against the early antigens E2, E6 or E7 as well as the late antigen, L1, are induced. However, difficulties in the quantitation and characterization of the T-cell response, precludes the interpretation of these data with respect to the strength and type of immune response in relation to the state and outcome of infection.

3.2. Detection of T-cell immunity, ‘new-style’

More recently, alternative methodologies that are more suitable for charting HPV-specific T-cell responses have been applied. Antigen-specific CTL are visualized through binding of their T-cell receptors with fluorescently labeled MHC class I-peptide tetramers. Alternatively, antigen-specific T-cells (CTL and Th-cells) are detected via antigen-induced cytokine production as measured by intracellular cytokine staining or ELISPOT. These techniques allow enumeration of antigen-specific T-cells, direct in freshly isolated PBMC or after a few days of culture.

HPV-peptide loaded HLA-A*0201 tetramers were used to measure the frequency and reactivity of HPV16 E7_{11-20}-specific CTL in patients with HPV16+ CIN III lesions (Youde et al., 2000). Interestingly, about half of the patients showed E7-specific CD8+ T-cell frequencies in the range of 1/1260 to 1/4000 (Youde et al., 2000). This is well within the range of HLA-A*0201 restricted CTL responses against common viruses, like influenza virus (Dunbar et al., 1998). In addition, HLA-A*0201-restricted HPV16 E6_{29-38}-specific CD8+ T-cells were found in 2/10 cancer patients
following short-term in vitro stimulation (Evans et al., 2001). The use of HLA tetramers for the analysis of HPV-induced CTL immunity has its limitations. The construction of HLA tetramers requires full knowledge of the minimal CTL epitope. Furthermore, each HLA-restriction element necessitates the construction of different HLA-peptide tetramers. Up to now, only HLA-A*0201-restricted HPV-specific CD8+ T-cells have been analyzed. Importantly, in spite of the superior quality of HLA tetramers to enumerate antigen-specific CD8+ T-cells, tetramers do not directly discriminate between antigen-experienced CD8+ memory T-cells and antigen-specific naïve CD8+ T-cells (Lee et al., 1999; Pittet et al., 1999). Full characterization of antigen-specific CTL, therefore, involves the use of other cell-surface markers in combination with techniques to determine cytokine production.

We prefer to analyze HPV-specific T-cell immunity by the ELISPOT assay. The enumeration of antigen-induced cytokine producing T-cells directly identifies antigen-experienced T-cells, also when present at low frequencies (Di Fabio et al., 1994). Furthermore, the flexibility offered by this assay with respect to the detection of both HPV-specific CTL and Th-cells—restricted by different HLA types—permits the analysis of T-cell reactivity in all subjects. The IFNγ ELISPOT assay has been used successfully to detect virus-specific CD8+ T-cells (Herr et al., 1998; Larsson et al., 1999; Lechner et al., 2000; van Baarle et al., 2001) including HPV-specific CTL (our unpublished observations). The detection of memory T-cell activity against common recall antigens requires a 3-day stimulation protocol (van der Burg et al., 2001b) and application of this ELISPOT assay revealed HPV-specific IFNγ-producing memory Th-cells against both HPV16 E6 and E7 in about 30% of HPV16+ CIN III and cervical cancer patients (van der Burg et al., 2001b and our additional unpublished data). However, additional studies are necessary to analyze the presence of HPV-specific Th-cells producing other (type 2) cytokines. In our view, the complete evaluation of the HPV-specific memory T-cell response necessitates the combination of high sensitivity, stringent criteria and flexibility to screen for CTL and Th-reactivity as well as different cytokines with e.g. the quantitative ELISPot technique. Such an analysis would allow a reliable comparison of this response between groups of healthy subjects and patients with HPV16-related disorders of the uterine cervix.

4. HPV16-induced T-cell immunity in healthy subjects

Several of the earlier studies revealed E6 and/or E7-specific Th-reactivity in 0–47% of the healthy individuals tested (de Gruijl et al., 1998, 1996; Kadish et al., 1994; Luxton et al., 1996; Tsukui et al., 1996). Although it is still unclear whether these data really reflect HPV-specific immunity, the existence of HPV-specific immunity in HPV DNA-negative individuals is appealing because it may reflect the remnants of an effective immune response against prior infection. The high annual incidence of HPV16 (Ho et al., 1998; Koutsky, 1997) as well as the prevalence (7%) of sub-clinical HPV16 in women with microscopically and cytologically normal cervixes (Lambropoulos et al., 1994), defines the healthy population as a group that includes individuals who have effectively dealt with HPV infection. The presence of HPV16 L1-VLP-specific antibodies reflects prior and ongoing infection with HPV16 and the levels of these antibodies are stable over time (af Geijersstam et al., 1998; Konya and Dillner, 2001). HPV16 L1-specific IgG antibodies have been detected in 19% of HPV DNA-negative college women (Viscidi et al., 1997) pointing at prior HPV16 infection in a substantial number of healthy subjects. Interestingly, Youde et al. detected high frequencies (<1/5000) of HPV16 E711–20-tetramer positive CD8+ T-cells in 4/10 healthy individuals directly ex-vivo (Youde et al., 2000), which may explain why this peptide was found to be so highly immunogenic (Ressing et al., 1995). But as stated previously, phenotyping and in-depth functional analysis of these CD8+ T-cells is now required to unravel the role of these cells in HPV infection.

The availability of a highly sensitive assay to detect functional HPV16-specific memory T-cells prompted us to re-examine HPV16-specific Th-
immunity in healthy subjects. Incubation of freshly isolated CD45RO+ PBMC, representing the memory T-cell fraction, with HPV16 E2-peptides for 10 days, demonstrated the presence of HPV16 E2-specific Th-cells in 6/10 healthy subjects (de Jong et al., 2002b). Moreover, HPV16 E6-specific Th-cells were detected in ~50% of healthy subjects by a 3-day IFNγ ELISPOT (our unpublished data). Thus, both HPV16 E2- and HPV16 E6-specific IFNγ-producing Th-cells are frequently found in subjects without clinical signs of infection. This partly confirms the study of Kadish et al. who longitudinally studied E6/E7-specific Th-cells in 6/10 healthy subjects (de Jong et al., 2002b). Moreo...

5. Vaccination against different backgrounds of HPV16-specific T-cell immunity

Priming of HPV-specific immunity exclusively depends on cross-presentation of viral antigens by epithelial resident dendritic cells (Langerhans cells; LC), which are specialized in the transfer of antigenic signals to the draining lymph nodes and orchestration of T-cell responses. An important factor that is likely to play a role in the activation and make-up of HPV-specific immunity, is the cytokine-environment in which LC are conditioned (reviewed in Offringa et al., 2002). Keratinocytes secrete immunoregulatory factors such as transforming growth factor beta (TGF-β) and interleukin 10 (IL-10) which play a role in the differentiation of LC as well as in the retention of LC in the epidermis (Jakob et al., 2001). Under certain conditions keratinocytes can secrete pro-inflammatory cytokines (Kondo, 1999). Provided that these signals are of sufficient strength to endow LC with the full capacity to activate antigen-specific T-cells, effective HPV-specific immunity will ensue.

In a minority of cases viral infection persists and CIN may develop. Progression of these CIN lesions results in the enhanced expression of the immunosuppressive cytokines IL-10, TGF-β1 and PGE-2 (Giannini et al., 1998; Mota et al., 1999; Sales et al., 2001) and loss of the pro-inflammatory cytokine TNF-α (Mota et al., 1999). If the immune system encounters HPV-derived antigens during the period that immunoregulatory signals dominate pro-inflammatory signals, activation and maturation of LC will be inhibited. As a consequence HPV-specific immunity is likely to be too late and of insufficient strength. Against this background, vaccines incorporating strong inflammatory signals probably result in the boosting of strong HPV-specific Th1/CTL immunity.

Activation of DC or LC in the presence of IL-10, TGF-β, or PGE2 was shown to result in the APC that mainly induces Th2-type immunity (Bellinghausen et al., 2001; King et al., 1998; Liu et al., 1998). Priming of HPV16-specific immunity by Th2-type polarized DC may, therefore, result in a relatively ineffective Th2-type T-cell response against solid tumors. Boosting of this type of HPV-specific immunity by vaccination may induce a paracrine loop between Th2 cells and APC that will further incapacitate the induction of Th1/CTL-type immunity (Ria et al., 1998). In this situation, vaccines may need to be administered together with potent adjuvants that can convert Th2-type into Th1-type immunity, such as ODN-CpG (Zimmermann et al., 1998).

Alternatively, the lack of pro-inflammatory signals may result in LC that were not properly matured. Antigens will then be presented to T-
cells, but not in an appropriate context. This may result in T-cell tolerance (reviewed in Offringa et al., 2002). DC that have been activated in a non-inflammatory context displayed a strongly decreased ability to activate antigen-specific T-cells and even resulted in hypo-responsiveness of CD4+ T-cells (Rea et al., 2000). Furthermore, the antigen-specific interaction between such anergic T-cells and DC can suppress the activation of both naive and antigen-experienced T-cell responses (Vendetti et al., 2000), by downregulating the expression of costimulatory molecules on immature DC as well as initiation of apoptotic cell death of mature DC (Frasca et al., 2002). In theory, the presence of such HPV-specific T-cells may prevent the induction of effective Th1/CTL-mediated anti-tumor immunity by vaccines. In addition, improper vaccination may further enhance HPV-specific suppressor activity in this situation.

At present several vaccines have been tested in phase I/II clinical trials. They include peptide-based vaccines (Muderspach et al., 2000; Ressing et al., 2000; Steller et al., 1998), fusion-proteins (de Jong et al., 2002a; van der Burg et al., 2001a), antigen-pulsed DC (Adams et al., 2002) or recombinant vaccinia viruses (Adams et al., 2001; Borysiewicz et al., 1996). In general, these vaccines proved to be safe. Vaccination of cervical cancer patients resulted in the detection of an occasional vaccine-induced T-cell response against HPV (Adams et al., 2001; Borysiewicz et al., 1996; Ressing et al., 2000; Steller et al., 1998). As the patient group treated consisted of late stage cervical cancer patients, the lack of good responses was attributed to tumor-related immunosuppression. Furthermore, Th2-type immunity seems to prevail in such patients (al-Saleh et al., 1998; Clerici et al., 1998). Interestingly, however, patients vaccinated with a recombinant vaccinia virus were able to mount vaccinia-specific immunity (Borysiewicz et al., 1996). Furthermore, injection of a peptide-based vaccine that contained two HPV16 E7 CTL epitopes and one non-related universal Th-epitope (PADRE), resulted in the induction of PADRE-specific Th-cells but not HPV16-specific CTL (Ressing et al., 2000). Notably, these vaccines were able to enhance HPV-specific immunity in VIN III patients with pre-existing Th1-type T-cell immunity (Muderspach et al., 2000, and our unpublished observations). Thus, in spite of immunosuppression, end-stage patients are able to mount immunity to non-HPV related vaccine elements. This prompts the question why they failed to do so against the HPV-derived epitopes and if this was related to a particular type of pre-existing immunity.

Finally, a new breed of powerful vaccines is now ready for clinical trials. In animal models, these therapeutic vaccines possess the capacity to protect against tumors not only in a prophylactic setting but also in a minimal residual disease type of setting (van der Burg et al., 2001a), and even in a therapeutic setting (Chu et al., 2000; Daemen et al., 2002; Gunn et al., 2001; Lamikanra et al., 2001; Velders et al., 2001; Zwaveling et al., 2002). In clinical trials of these vaccines, the characterization of pre-existing HPV-specific immunity, as well as of changes induced by the vaccination itself are crucially important to identify those vaccines that are capable of inducing the most powerful protective T-cell responses and, if necessary, able to overrule pre-existing deleterious HPV-specific T-cell responses including, Th2-type and T-regulatory responses.

Acknowledgements

Our work as discussed in this manuscript was supported by grants 99-2024 and 00-2200 (R.O. and C.J.M.M.) of the Dutch Cancer Society, and by a grant of the Cancer Research Institute (S.H.vdB).

References


responses in patients with advanced cervical cancer using autologous dendritic cells (DC) pulsed with autologous or allogeneic cervical tumor cell lysate, submitted for publication.


cervical cancer among women with, or at risk for, HIV infection. Int. J. Cancer 82 (3), 334–337.


