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Cervical Cancer

The Potential Role of Human Papillomavirus (HPV)-Specific Vaccines in Prevention and Treatment

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Abstract

Worldwide about half a million new cases of cervical cancer occur each year. The incidence is about three times higher in resource-poor countries compared with more developed countries. The disease is reasonably well controlled in countries where routine cervical cytology for detection of premalignant precursors (cervical intraepithelial neoplasia; CIN) is available.

Since the causal link between infection by so-called high-risk types of human papillomaviruses (HPV 16, 18 and others) and cervical cancer has been firmly established, the development of virus-specific vaccines has become a major activity both in the academic and corporate sectors. During the natural history of cervical cancer, there are different possible windows for vaccination: (i) prevention of infection that is conferred by neutralizing antibodies can be achieved by immunization with virus-like particles (VLP). Clinical trials with HPV 16 VLPs in humans demonstrated the safety and immunogenicity of the vaccine. Analysis of clinical endpoints such as prevention of infection, CIN and ultimately cervical cancer will require a longer follow-up time; (ii) HPV-associated cervical diseases can also possibly be prevented by postexposure vaccination. As persistent HPV infection appears to be a prerequisite for the development of a malignant tumor, the viral proteins expressed during this state (e.g. E6, E7) are potential targets for cytotoxic T-cell responses to eliminate the infection. Since the target population for this vaccination strategy will consist of mostly young sexually active women that are at risk for reinfection or are potential carriers of infectious virus, it seems to be reasonable to induce a protective immunity *via* neutralizing antibodies as well. Chimeric virus like particles (CVLP) containing both the L1 (with or without L2) and E7 proteins are promising tools to achieve this goal; and (iii) treatment of cervical cancer by HPV E6/E7-specific immune therapy will most likely only be successful as an adjuvant strategy along with other therapies. On the other hand, based on the data from the first clinical trials, the option of curing precursor lesions by HPV-specific vaccination is considered promising.

Papillomavirus-induced diseases are in most instances self limited benign proliferations, (i.e. warts of the skin or mucosal surfaces). Only some lesions will develop into malignant tumors at low frequency and after a long time. In humans, cancer of the uterine cervix arising from cervical intraepithelial neoplasia (CIN) is numerically the most relevant malignancy among such diseases. The papillomavirus family consists of more than 100 members, of which 86 are specific for humans and 16 for animal species (e.g. cattle, dogs or rabbits; for review see de Villiers[1]). Within a given species, classification of papillomaviruses is based upon the nucleotide sequences of the circular DNA genome (about 8000 base pairs). Papillomaviruses share remarkable similarities in virus morphology, genome structure (eight early and two late genes), and biological properties (growth exclusively in differentiating epithelia). Yet the lesions that are arising as a consequence of an infection by the individual human papillomavirus (HPV) types can differ in location, clinical appearance (exophytic warts, flat lesions), and natural history (benign vs exhibiting a potential towards malignant progression).

HPV are classified either according to their preferred tropism in skin or mucosa (cutaneous, e.g. HPV 1, 2, 4, or mucosal, e.g. HPV 6, 16, 18) or, according to their transforming potential as low- or high-risk types. Because the causal association between cervical cancer and infection by particular HPV types (16, 18, 31, 33, 45,

and others[2]) has been firmly established through experimental studies and epidemiological surveys (for reviews see IARC working group[3] and zur Hausen[4]), the use of virus-specific diagnostics and antiviral strategies in medical practice is widely investigated. Whereas virus-specific small molecules have not yet passed the state of laboratory experiments,[5,6] the development of virus-specific vaccines is fairly advanced and the first results from clinical trials have been disclosed (see section 6). Other cancers are also associated with infection by high-risk HPV types, although here the link is less consistent (e.g. squamous cell cancer of the head and neck) or less significant in terms of total numbers (e.g. cancer of the penis and vulva). Obviously, the demand for an HPV-specific vaccine varies according to the severity of the disease and clinical importance. Today, the major focus regarding an HPV-specific vaccine(s) is towards prevention and therapy of cervical cancer and its precursors.

1. Epidemiology of Cervical Cancer

1.1 Demography

The age-standardized incidence of cervical cancer throughout the world varies between 2 (Saudi Arabia) and 90 (Haiti) per 100 000 women each year, and is 3 times higher in less developed compared with more developed countries.[7,8] Worldwide, approximately 500 000 women develop cervical cancer each year. Each year about 350 000 women die from the disease. The incidence of adenocarcinoma, especially in younger women, has been increasing over the last decade.[9-13] Changes in sexual behavior and inefficiency of cervical screening to detect adenomatous lesions may explain this phenomenon. The incidence of cervical precancerous lesions is approximately 100 times higher than for invasive cancer. Seventy-seven percent of cervical cancers are of squamous, 11% of adenomatous, 2.5% of adenosquamous, and the rest of rare cell types.[14]

1.2 Risk Factors

The presence of HPVs is essential for cervical carcinogenesis. Since only very few HPV-infected women develop cervical cancer, additional risk factors that act independently of or interactively with HPV may be necessary. For the majority of epidemiologically identified factors, their mode of action is unknown.[15]

Invasive cervical cancer is rare in women <20 years of age. There is one age peak at 40 and another one at 60 years of age.[16] An increase in incidence and mortality rate was only reported for African-Americans. For the detection of HPV, there is an age peak at 25 years then a continuous decrease with increasing age.[17,18] This is explained by the acquisition of HPV following sexual debut and decreasing exposure with increasing age.[19] However, after menopause, a slight increase in the HPV detection rate has been shown.[20] The association among the number of sexual partners, early cohabitation and risk for cervical cancer disappears when controlled for HPV.[21-23] In contrast, multiparity is a risk factor for cervical cancer also in HPV-positive women.[24,25] The association between the number of full-term pregnancies was significant for squamous cell carcinoma, but not for adeno- or adenosquamous carcinoma.[24] High-risk sexual behavior of the male partner may partly explain the geographic differences in the incidence of cervical cancer.[26,27] Incidence and persistence rates of HPV infection in young men are similar to women.[28] Male circumcision reduces the risk of genital HPV infection in high-risk men and the risk of cervical cancer in their female partners.[29]

Cervical cancer is found most commonly in women of low socioeconomic status.[30,31] However, other covariables such as bad nutrition, lack of screening, and genital infections confound this association.[32,33]

No clear association can be found between herpes simplex virus (HSV) 2 seropositivity and cervical cancer when controlled for HPV status.[32,34-37] For chlamydia trachomatis infection, the data on association are controversial.[32,37-41]

Several studies show an HPV-independent effect of smoking, whereas other studies did not find an association when HPV was taken into account.[21,22,35,42,43] The excess risk for women who have ever smoked among those who are HPV-positive is stronger for current smokers than for ex-smokers.[44] Regression of CIN was observed when women stopped smoking.[45] The molecular basis of a possible association is unclear: smoking may be directly genotoxic or modulate immune response.[46]

Studies that evaluate an association between long duration of oral contraceptive (OC) use and cervical cancer show conflicting results.[3,22,42,47] A recent analysis of pooled data from eight studies of HPV DNA-positive patients and control individuals showed a 4-fold increased risk for cancer in those who had taken OCs for ≥10 years.[48] There may be a stronger association between long-term OC use and the development of adenocarcinoma.[49,50]

Women with CIN have lower serum levels of antioxidants and folic acid.[51-53] However, not all studies confirmed these results when taking HPV status into account.[54-56]

An increased incidence of CIN or invasive cancer has been reported in family members of patients with CIN or cancer.[30,40,57,58] This may in part be explained by an association between HLA status and the risk for CIN.[59-61] HLA DAP1* 1301 may be protective for cervical cancer. In addition to class II alleles (DR, DQ, DP), class I alleles (A, B, C) may also be important. It is unclear whether variations of HLA associations depend upon HPV variants (see section 1.4.2).

Experimental evidence indicates that Arginin/Prolin polymorphism of codon 72 of p53 determines the effectiveness of HPV 16 or HPV 18 E6 proteins to degrade p53 *in vitro*.[62] It is discussed controversially as to whether or not this correlation also holds true *in vivo*.[62-66]

CIN is more frequently diagnosed in iatrogenically immunosuppressed women.[67-70] HIV-positive women have an increased risk for HPV infection and CIN and the risk is linearly associated with a decreasing number of CD4+ cells.[71-76] It is not clear whether HIV-induced immunosuppression leads to reactivation of latent HPV infection, to increased risk of reinfection, or both. High prevalence of novel HPV genotypes, presence of multiple infections, and high viral load (see section 1.4.2) in HIV-positive women indicate that activation of latently present HPV types may be the most important biologic phenomenon in immunosuppression.[61,77] Prevalence of HPV type 16 is only weakly associated with immune status in HIV-positive women.[78] This may indicate that HPV 16 is effective in avoiding immune surveillance. It remains unclear whether or not progression to cancer is faster in HIV-positive patients.

1.3 Natural History of Human Papillomavirus (HPV)

Infections by genital HPVs are primarily transmitted by sexual contact[79-88] but perinatal, digital, and oral transmission have also been described.[89-96] The prevalence of HPV DNA in cervical smears in young women is high and usually not associated with clinical symptoms.[97] The median duration of HPV DNA detection is 8 months[98] and 80% of infections regress spontaneously. Women with persistent infection can, under rare circumstances, develop invasive cancer. Since women with persistent HPV infection are treated for CIN, the risk for developing invasive disease is not known. Persistence is associated with older age, presence of high-risk HPV types, and infection with multiple HPV types.[98] HPV 16 infections seem to either progress or regress but, in contrast to infections by other HPV types, do not persist without progression.[98,99] Low-grade may progress to high-grade CIN,[100] but development of high-grade precancer without pre-existing low-grade lesions has also been reported.[52]

1.4 Testing for HPV

Diagnosis of HPV infection is most commonly performed by detection of the viral DNA. Today, the three test systems most commonly being used are: the MY09/11 and the GP5+/6+ consensus primer polymerase chain-reaction (PCR) technologies[101,102] and the second-generation of the Hybrid Capture system, which is currently the only system commercially available. With these methods, approximately 20 high-risk HPV types can be identified. All three tests can be automated. Comparing MY09/11 and Hybrid Capture II good agreement is found (Kappa indices between 0.59 and 0.7).[103] A comparison of the MY09/11 and GP5+/6+ system showed very good agreement with a Kappa index of 0.83.[104]

HPV testing has been evaluated: (i) for screening; (ii) for triaging patients with abnormal cytologic findings or low-grade CIN; and (iii) for detection of recurrence following conization. A considerable number of studies have been performed, which used different techniques of cytology, different morphologic classification systems, and different HPV detection tests. This has to be kept in mind when comparing results from the various studies.

1.4.1 Screening

HPV testing may be used in addition to or instead of the cervical Papanicolaou (PAP) smear. Various studies showed increased sensitivity for detection of CIN II or III when HPV testing was combined with cytology[105-107] (for review see Cuzick et al.[108] and Mandelblatt et al.[109]). In two recent studies in which results were corrected for status of disease in test negatives and for noncompliance of test positives, a significantly higher sensitivity for HPV testing compared with cytology was shown[15,110,111] (table I). Also, the negative predictive value of HPV testing was superior to cytology, whereas specificity was significantly lower. These findings have been confirmed recently in a 10-year prospective study; the authors reached the conclusion that in Western countries which favor reassurance of safety, HPV screening may complement or eventually supplant cytologic methods as a primary screening.[112] This recommendation will only be made when the cost effectiveness of HPV testing in screening has been shown,[113] which has been done in one simulation model so far: comparing 18 different screening strategies, HPV plus cytologic screening every 2 years appeared to save additional years of life at reasonable costs compared with cytology alone.[109]

Since the establishment of cytologic screening programs may be difficult in resource-poor settings, HPV testing on self-collected vaginal samples may be an alternative. Different studies showed that sensitivity for detection of CIN II or III or cancer was similar for self collected samples evaluated for high-risk HPV and PAP smears collected by gynecologic examination.[113,115,116] Such a test may not only be implemented in so-called Third World countries but may also be used to reach women who do not wish to undergo a gynecological examination.

1.4.2 Atypical Papanicolaou (PAP) smears

Detection of high-grade CIN or cancer in women with the cytologic diagnosis of atypical findings or CIN I by detection of high-risk HPV has been evaluated in various studies (for review see Cuzick et al.[108] and Kim et al.[117]), the largest of which have been conducted in patients with the diagnosis of atypical squa-

Table I. Human papillomavaris (HPV) DNA detection in screening with bias-corrected estimates for sensitivity and negative predictive value (reproduced from Schneider et al.,[114] with permission)

Reference	Number of women	Mean age (y)	HPV detection method	Prevalence of HPV (%)	Prevalence of CIN II/III or CA (%)	Sensitivity[a] (%)		Negative predictive value[a] (%)	
						HPV	cytology[b]	HPV	cytology[b]
Schneider et al.[110]	4671	35	GP 5+/6+	7.8	2.4	89.4	20	99.6	97.54
Ratnam et al.[111]	2098	30	HC I/HC II	10.8	1.6	68.1	26.8	99.1	98.1

a For CIN II/III or carcinoma.

b For any CIN.

CA = cancer; **CIN** = cervical intraepithelial neoplasia; **GP** = primer polymerase chain-reaction technology; **HC** = hybrid capture; **HPV** = human papillomaviruses.

mous cells of undetermined significance (ASCUS). These studies showed that the sensitivity and positive predictive value of HPV testing was superior to cytology.[118,119] HPV testing was cost effective in one of these studies,[118] which was confirmed by computer-based modeling when comparing reflex HPV DNA testing with three other strategies.[117] Similar results were described in patients with abnormalities in the columnar epithelium, atypical glandular cells of undetermined significance (AGUS).[120] HPV testing using the Hybrid Capture II system for triaging women with low-grade CIN proved not to be useful because of the high prevalence of high-risk HPV positives in this group.[119]

The majority of low-grade CIN regress spontaneously.[121,122] Therefore, biologic markers that predict regression or progression would be clinically extremely useful. Repeated detection of high-risk HPV DNA is associated with recurrent or progressing CIN.[52,123-125] Women with a high load of HPV 16 DNA have a 60 times increased risk for the development of CIN III compared with women who are HPV 16 negative.[126] The effect of viral load may differ for variant HPV types. Using a PCR protocol that discriminates between HPV RNA from episomal and integrated genomes, it was shown that only 5% of CIN II, 16% of CIN III, but 88% of invasive cancers contain integrated HPV DNA molecules.[127] Genetic variants of the different HPV types vary in <3% of their DNA base sequences. Unlike with the corresponding HPV prototype, different variants – especially in the E6 gene and the long coding region of HPV 16, have been defined.[128-130] In different ethnic groups, different HPV variants are associated with risk for cancer.[131-133]

1.4.3 Follow-up After Cervical Intraepithelial Neoplasia (CIN)

Following conization, 1 in 1000 patients per year experience recurrent cancer, which accumulates to an incidence of 1% after 10 years.[134] Several studies show that persisting or recurrent CIN can be detected via the presence of high-risk HPV DNA with higher accuracy than cytology.[113,135]

2. Cervical Precancer

2.1 Diagnosis and Classification

Cervical precancer is not associated with symptoms. Anogenital condylomata may precede or accompany CIN but are not specific for the presence of cervical precancer. Therefore, there is no clinical sign or symptom that is reported by the patient and that may lead to proper diagnosis.

The gold standard for diagnosis of precancer of the uterine cervix is histologic evaluation. Various classification systems are in use.[100,136,137] In Europe, the dysplasia-carcinoma *in situ* concept is most popular.[137] The concept of CIN was defined in

1973.[100] In 1988, the Bethesda classification system was introduced replacing CIN by squamous intraepithelial lesion (SIL) and discriminating low-grade from high-grade SIL.[136] Although inaugurated for cytologic classification, the SIL system was later adapted for histologic classification.[138] There is a high inter- and intraobserver variability for low grade disease between different investigators.[139-141] Differentiation between low grade and high grade disease may be facilitated by the inclusion of specific markers such as p16.[142]

Colposcopy is the best technique to identify the location of ectocervical lesions and direct punch biopsy forceps to the area with the most severe histologic changes. Application of 5% acetic acid and 3% iodine solution allows the identification of an atypical transformation zone that may contain acetowhite epithelium associated with mosaicism or punctuation. In addition, leucoplakia or, in the case of cancer, atypical vessels can be identified. Lesions can be graded according to their color, surface-pattern, distance of the capillaries, demarcation of the lesion, and uptake of iodine. Using these grading criteria enables the colposcopist to take biopsies and define the severity of the lesion according to the histologic findings (figure 1).

For cytology, cells are taken from the squamocolumnar junction and the endocervical canal with the aid of different collection devices such as spatules, broom, or brush. The cellular material is either directly spread onto a glass slide or first suspended in a specific medium. Microscopic evaluation is then conducted in order to identify dyskaryotic or cancer cells. For classification, either the modified system according to Papanicolaou[143] and Soost et al.[144] or the Bethesda system[136] is used.

2.2 Treatment and Prognosis

When biopsy confirms the presence of high-grade precancer (CIN II or III), the lesion is either excised or ablated. In ablative techniques, only part of the tissue is examined when biopsies have been given priority. This may occur in up to 1% of patients.[145-148] Therefore, destructive techniques such as CO_2 vaporization or cryotherapy should be used in lesions that are purely of ectocervical location and preferentially of low-grade potential.[149-151] Excision of lesions is mainly done by electrosurgery[145-148] or CO_2 laser.[152] Both techniques allow significantly more preservation of cervical tissue compared with cold knife conization.[153,154] This may have positive effects on fertility,[155,156] operation time, blood loss, and postoperative complications.[157]

Noninvasive methods of treatment with interferon or other immune modulators have shown some effect in various studies but are not integrated in clinical practice so far.[158-161]

Ninety-five percent of patients treated with excisional therapy are cured. In 5% of patients persistence or recurrence of disease can be observed, which is closely associated with the presence of positive margins at the time of conization. Invasive cancer is observed in 1 in 1000 women per year treated for CIN III by conization.[134] Therefore, patients with a history of CIN III and conization should be followed up closely.

3. Diagnosis, Treatment, and Prognosis of Invasive Cervical Cancer

3.1 Diagnosis

In patients with invasive cervical cancer, vaginal bleeding is the most common symptom. In advanced disease, vaginal discharge caused by necrotic tumor, loss of weight, and/or symptoms associated with obstruction of the ureter and/or bowel may be observed.

Gynecologic bimanual evaluation is the most important examination for defining the extent of disease. In particular, infiltration of the tissue surrounding the cervix (parametrium) can be performed by gynecologic examination. By colposcopic examination, atypical vessels can be identified if the tumor involves the ectocervix. Tumors with an exclusive endocervical location can only be diagnosed by biopsy, curettage, or conization.

The gold standard for the diagnosis of cervical cancer is histologic verification. This is done in clinically apparent tumors by colposcopically guided punch biopsy. In the case of early invasive yet clinically not apparent cancer, conization has to be performed in order to define the extent of the disease.

3.2 Staging

Since the majority of cervical cancers are treated by radiotherapy, this tumor is staged clinically in the International Federation of Gynecology and Obstetrics (FIGO) system. The tumor, node, metastasis classification (TNM) system can be used in patients who undergo surgery[162] and allows better correlation with prognosis compared with the FIGO system. Cervical cancer can spread by the following: (i) direct continuing growth into the stroma of the cervix, corpus of the uterus, vagina, parametrium, and/or adjacent organs such as bladder or rectum; (ii) lymphogenic spread into the pelvic and/or para-aortic lymph nodes; (iii) hematogenic spread into the adjacent soft tissue of the pelvis, lungs, liver, and/or skeleton; and (iv) intraperitoneal spread by continuous growth through the peritoneal lining of the cervix. For staging according to FIGO, in addition to the clinical evaluation, an intravenous urogram, contrast enema, and radiography of lungs and skeleton are allowed. Cystoscopy, rectoscopy, and endocervi-

cal curettage can be performed. A computed technology CT scan, lymphangiogram, sonography, magnetic resonance imaging (MRI), scintigraphy, and laparoscopy are optional. However, the findings of these optional examinations cannot influence FIGO staging: the stage, which is defined by primary examination, cannot be changed by later findings.

3.3 Therapy

An interdisciplinary board of pathologists, medical oncologists, radiotherapists, radiologists, and gynecologists should be set up prior to the selection of therapy. For patients with early disease (stage Ia or Ib1), surgery is the treatment of choice. Stage Ib2 and stage II can be treated by either surgery and/or radiochemotherapy. Patients with large but localized tumors may benefit from adjuvant chemotherapy prior to surgery. For primary treatment of advanced cervical disease (stage III and IV), a combination of chemo- and radiotherapy is the standard of care.[163-165] This also may be true for adjuvant treatment following primary surgery in patients who show an extension of the disease to the parametrium and/or lymph nodes.[166] For patients with stage IV disease, palliative treatment with radiotherapy and/or chemotherapy is performed.

Abdominal radical hysterectomy with pelvic lymphadenectomy is the standard care of surgical treatment. In tumors at high risk for lymph node metastasis, para-aortic lymphadenectomy is included.[167] Laparoscopic lymphadenectomy in combination with vaginal radical hysterectomy has been performed recently.[168] A combination laparoscopic and vaginal approach allows preservation of fertility by radical trachelectomy in patients with small tumors who are at low risk for lymphatic spread.[169] Several types of radical hysterectomy have been defined; the extent of resection of parametrial tissue is tailored according to the extent of disease.

Radiotherapy in primary treatment is performed as a combination of tele- and brachytherapy. Brachytherapy is usually performed by after loading technique and allows intracavitary application of a high-, medium- or low-dose rate. Usually 60 Grey are given externally to the whole pelvis using various techniques. In combination with brachytherapy up to 80 Grey can be given to a specific point 2cm lateral and cranial to the vaginal fornix.

With respect to chemotherapy a number of agents have been defined that show, when given as monotherapy, a response rate of >15%.[170] The most effective substances are cisplatin, ifosfamide, fluorouracil, and taxol. Combined with radiotherapy, either cisplatin alone or in combination with fluorouracil is given.

3.4 Prognosis

HIV status, comorbidity, diabetes, thrombocytosis, hemoglobin level, blood pressure, temperature, and nicotine use have been evaluated as independent prognostic factors.[171-174] The data are controversial and no clear independent association has been found for these factors (for review see Rubin and Hoskins[175]). There is a linear correlation between increasing age and decreasing 5-year survival.[176]

Several tumor-associated risk scores have been defined, which allow estimates of prognosis that take into account the different tumor-associated factors.[171-174] Lymph node status is the most reliable prognostic factor: patients with positive para-aortic and/or pelvic lymph nodes have a significantly higher recurrence rate than patients with negative lymph nodes.[177] There is a linear association between the number of positive lymph nodes and recurrence and 5-year survival rate.[178] In patients with positive para-aortic lymph nodes, the survival rate is not >25% after 3 years.[179]

There is a linear correlation between FIGO stage and tumor involvement of lymph nodes. Tumor size and, more specifically, tumor volume are associated with local recurrence and systemic metastasis.[180] Tumor grading for squamous and adeno carcinoma is an independent prognostic factor: with decreasing differentiation the recurrence rate increases.[180] Tumor invasion in the cervical stroma is another independent prognostic factor for recurrence and survival.[181] Thus, continuing tumor growth seems to be an unfavorable prognostic factor.[182,183] Invasion of the lymphovas-

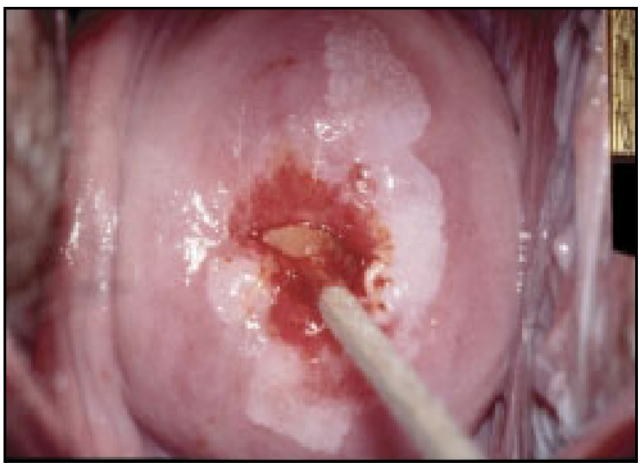

Fig. 1. Colpophotogram of a 25-year-old patient with negative cytology and detection of high-risk human papillomavirus (HPV) by polymerase chain reaction (PCR). There is an atypical transformation zone with a semicircular acetowhite lesion showing mosaicism. The color of the lesion is opaque and the border sharp and elevated. The squamo-columnar junction is visible. A biopsy was taken at 9.00am and showed cervical intraepithelial neoplasia (CIN) III. The lesion was removed by diathermy loop conization. Since then the patient has been HPV-negative and without evidence of disease (6 years). This patient would have been a candidate for therapeutic vaccination (if already available). However, immune therapy versus conservative treatment needs to be compared in terms of cost-effectiveness and patients' compliance.

cular vessels is an early sign for increased risk and precedes detection of tumor cells in lymph nodes.[184] Infiltration of blood vessels is associated with a 5-year survival rate of <30%.[182] Microscopic invasion of the parametrium is an independent negative prognostic factor.[185] It is controversial as to whether adenocarcinoma has a different prognosis compared with squamous cell carcinoma. Patients undergoing primary radiotherapy have a significantly lower survival rate if they are diagnosed with adenocarcinoma compared to squamous cell carcinoma.[186,187] Patients with small cell cancer, clear cell cancer, and undifferentiated large cell nonkeratinizing squamous cell cancer have a worse prognosis than patients with other histologic types.[188,189]

Squamous carcinoma cell antigen levels in squamous cancer, carcino embryonal antigen, and CA 125 in adenocarcinoma, and markers such as prolactin, lactate dehydrogenase, urokinase-type plasminogen activator, and immunosuppressive acid protein A have been evaluated in their association with prognosis but are of limited clinical value.[175] DNA cytometry for assessment of ploidystatus, S-fraction, and expression of cytokeratines have also been investigated, but their usefulness has so far not been established.[175]

4. Immune Biology of Papillomavirus Infection

Following natural infection, papillomaviruses are not very immunogenic since their replication is confined to an immunological privileged site (i.e. the terminally differentiated keratinocytes). However, there are several lines of evidence suggesting that the immune system plays an important role in controlling papillomavirus infections, although it is unclear by which event(s) the immune responses are triggered during the natural course of infection: (i) whereas antibodies to virus particles develop in only a fraction of cases of proven incident HPV infection,[190] a humoral immune response does correlate with persistent infection and clinically visible disease.[191-194] Individual HPV types seem to represent antigenically distinct serotypes[195] indicating that during evolution the immune system has been driving the diversity of these viruses; (ii) T-cell responses are not found in all HPV-infected individuals (reviewed by Konya and Dillner[196] and Man[197]) but the development of a disease following virus infection seems to be incompatible with a Th1-type immune response.[198] Local and systemic HPV-specific T-cell responses and the appearance of Th1-specific cytokines correlate with regressing lesions;[199-203] (iii) immune modifiers that induce regression of genital warts in a proportion of patients were shown to induce different interferons,[204] whereas early proteins of some HPV types seem to suppress their expression;[205,206] and (iv) HPV infections are more likely to persist and less likely to respond to treatment in immunosuppressed individuals.[207,208]

Experiments with animal papillomaviruses in their natural hosts, (i.e. the cottontail rabbit papillomavirus [CRPV], the bovine papillomavirus [BPV], and the canine oral papillomavirus [COPV]) demonstrate that papillomavirus infections can be controlled by the immune system. Upon experimental inoculation, the animals develop papillomas with a similar time course and histopathologic features as during natural infection.[209,210] Efficient protection against tumor development or even induction of regression of established tumors was obtained when the animals were immunized with inactivated virions, recombinant structural or early proteins (for review see Breitbund and Coursaget[211]). Efficient protection was shown to be conferred by neutralizing antibodies directed against conformational epitopes. The most striking results have been obtained when virus-like particles (VLPs) were used for immunization. Upon expression in recombinant systems such as yeast, vaccinia- or baculovirus-infected cells, the VLPs assemble spontaneously from the major structural protein L1 together with, but also in the absence of, the minor capsid protein L2,[212] (reviewed by Schiller and Roden[213]). In numerous preclinical studies, VLPs of different animal and human papillomaviruses were demonstrated to induce high-titer L1-specific neutralizing antibodies in small laboratory animals and, in addition, protective immunity in their natural hosts (reviewed by Breitbund and Coursaget[211]). L1-specific serum antibodies were found to transudate into vaginal secretions following systemic vaccination, fulfilling the need of a local immunity in order to prevent mucosal HPV infections.[214] Alternatively, a strong mucosal, mostly immunoglobulin class A (IgA)-specific, antibody response was induced by intranasal immunization with VLPs.[215,216] L1-specific T-cell responses have also been demonstrated.[217,218]

Again from experiments with animal papillomaviruses in their natural host, there is evidence that a cell-mediated immune response directed against early viral proteins is critical for controlling the course of papillomavirus infections (for review see Breitbund and Coursaget[211]). In the analysis of HPV-specific tumor rejection, antigen research has focused on the oncoproteins E6 and E7 of high-risk types for cervical cancer (i.e. HPV 16 and 18). These proteins are prime candidates as targets for an immune therapy since their constitutive expression in tumors is indispensable for cell proliferation.[219-224] It was demonstrated by several studies in rodents that a protective or sometimes even therapeutic effect against the growth of HPV E6- and/or E7-positive syngeneic tumor cells can be induced by immunization with either one of these antigens (delivered as purified proteins plus the appropriate adjuvants, as recombinant viral or bacterial vectors, protein-derived peptides or with chimeric virus-like particles (CVLP; for

review see Gissmann et al.[225] and Da Silva et al.[226]). CVLPs contain heterologous sequences fused by either the L1 or the L2 proteins;[227,228] (for review including informative illustrations of [C]VLPs see Schiller and Lowy[229]). HPV 16 E7-containing CVLPs were shown to induce (L1-specific) neutralizing antibodies as well as E7-specific cytotoxic T cells, hence they are considered a suitable vaccine in a scenario where both prophylactic and therapeutic aspects are required.[227,228,230-232]

5. Vaccine Scenarios

5.1 Prophylaxis

Since genital HPV infections are in most instances venereally transmitted, vaccination should occur prior to first intercourse and, as with any sexually transmitted infection, must include men and women in order to reduce the virus load within the population. Among women there seems to be a general acceptance for a prophylactic HPV-specific vaccine aiming at the prevention of cervical cancer.[233,234] It was suggested that it might be helpful to incorporate into a cervical cancer vaccine a component against genital warts that affect both sexes (i.e. an HPV 6/11-specific vaccine) to further increase the incentive among men.[234-236]

Intramuscular injection of purified particles is expected to induce a systemic immune response and immunoglobulin class G (IgG) transudating through mucosal surfaces. Thus, cervical infection should be prevented although the antibody levels may vary during the menstrual cycle.[237] Alternatively, immunization at mucosal sites (e.g. intranasally) is also being considered, supposedly leading to production of secretory IgA. Mucosal delivery of the antigen can also be achieved via genetic immunization using naked DNA, mucosotropic bacteria (e.g. apathogenic *Salmonellae*[238]) or viruses as carriers for the HPV genes.[239] Topical application is of particular relevance for developing countries where the use of needles should be avoided and where there is need for a stable vaccine independent of a cold chain. The production of VLPs in plants can be considered, allowing for more economical manufacturing and eventually even the application as an edible vaccine.[240-242] From the animal experiments mentioned in section 4, no conclusions about the duration of a protective response against natural exposure can be drawn, hence the immunization scheme still needs to be worked out following the results of the initial human trials.

More than ten different HPV types were found to be responsible for the development of cervical cancer,[2] and there is only very limited cross-protection across HPV types.[195,243,244] Because of the plurality of cancer-related types, a 'cervical cancer vaccine' is therefore an unrealistic goal. Yet the four most prevalent types (HPV 16, 18, 31, and 45) account for approximately 80% of the cancer cases worldwide.[2] Hence, a vaccine comprising these four types may be an acceptable compromise between sufficient protection and reasonable expense in clinical development. On the other hand there are attempts to develop a cross-protecting vaccine based on L1 recombinants or exploiting the more broad reactivity of the L2 protein.[245-248]

Since persistent infection with high-risk types over several years is the prerequisite for developing a high-grade cervical dysplastic lesion,[98,125] postexposure vaccination is another option for the prevention of cervical cancer. The early viral genes are expressed during persistent infection.[219,222] Hence, it appears to be appropriate to induce a Th1-biased immune response directed against one or several of the early antigens. Based on the data from animal experiments, the E7 protein is the prime target in a number of vaccine projects. Other candidates include the E6 and E2 proteins, although an efficient immune response against the latter may actually select for E2-negative escape mutants. Silencing of E2 expression is thought to be one of the steps towards malignancy, as E2 is often deleted in tumor cells resulting in a constitutive expression of the HPV oncoproteins E6 and E7.[249]

Given the young age of women with persistent infection who would be eligible for postexposure immunization, a vaccine that elicits both T cells directed against early viral proteins and neutralizing antibodies is the most obvious approach in this situation. It is currently under discussion as to whether a combined prophylactic and therapeutic vaccine would be useful in the pre-exposure immunization scenario. There actually may be some individuals who have already been infected prior to onset of their sexual life. Second, and more important, sterilizing immunity may be difficult to obtain, especially for an extended period of time following vaccination. Therefore, it also appears to be advisable to induce a cellular immune response directed against persistent infection as a 'safety net'. Treatment of CIN can be considered as prevention of cervical cancer but it will be discussed here as an aspect of therapy.

Because of the long period between HPV infection and the development of cervical cancer, the reduction of its incidence cannot be used as a clinical endpoint in human trials. Consequently, surrogate parameters have to be considered (i.e. the induction of neutralizing antibodies and prevention of new virus infection and CIN).

5.2 Therapy

The ultimate goal of HPV-specific vaccination is a reduction in the incidence of cervical cancer. However, a significant worldwide effect of prophylactic vaccination would be visible only three

decades after the launch of such programs.[250] Consequently, attempts towards immune therapy must be made that will, if successful, show a much earlier obvious benefit. Even if less conclusive than in a prophylactic setting, the studies on animal papillomaviruses in their natural host also provide arguments in favor of a successful immune therapy against papillomavirus-induced tumors in humans (for review see Breitbund and Coursaget[211]). The development of first generation vaccines has focused on the early proteins E7 and/or E6 (discussed in more detail by Gissmann et al.[225] and Da Silva et al.[226]).

Because of the high spontaneous regression rate of low-grade CIN, HPV-specific immune therapy of intraepithelial neoplasia will most likely be restricted to high-grade lesions (see figure 1). It is generally believed that invasive cancer will be extremely difficult to treat by immune therapy only. It appears unlikely that the immune system is able to cope with the high burden of tumor cells, since they can exert immunosuppressive functions[251] and cause a complete or heterogeneous loss of HLA class I expression.[252,253] However, one can assume that HPV-specific therapy is helpful as an adjunct to standard treatment aiming to reduce the risk for relapse and improve quality of life. HPV-specific immune therapy of CIN appears promising, since the expression of class I molecules is less compromised within such lesions.[254,255]

A Th1-biased immune response is necessary for induction of 'antitumor' immunity[198] and consequently soluble proteins are *per se* insufficient as antigens. Suitable adjuvants are not readily available for use in humans (for more detailed discussion see Gissmann et al.[225]); hence, alternative means of antigen delivery have been explored. Immunization of HPV early proteins fused to peptides that direct the molecules into the major histocompatibility complex (MHC) class I pathway (e.g. the hsp65 heat shock protein of *Mycobacterium bovis*[256]) was shown to induce a CD8+ immune response in mice. Immunization with fusions between E7 (or E6 plus E7) and the L2 minor protein also induce good cytolytic T-lymphocyte (CTL) and T-helper responses, especially when applied in a prime boost scheme with recombinant vaccinia.[257] Use of long overlapping peptides or *ex vivo* loading of dendritic cells with proteins are other promising approaches that have been successfully explored in preclinical studies (for review see Da Silva et al.[226]). Delivery of early HPV genes through recombinant viral vectors is also under investigation.[258-260] The HPV E6/E7-positive vaccinia virus has already been tested in clinical trials (see section 6). If a vaccination strategy is promoted that depends upon the viral oncogenes E6 and E7, appropriate modifications of the DNA must be included. However, it is unclear whether point mutations at biologically important sites of the protein (e.g. the retinoblastoma protein binding site) provide a sufficient level of safety. One should consider including additional safety features such as the use of minigenes or shuffled sequences.[261]

In human trials, the clinical response can easily be measured by cytology or colposcopy and by the absence of detectable HPV DNA. The analysis of immunological parameters (HPV-specific cytotoxic T cells and T-helper cells) will provide additional information about the duration of a response and about a possible cross-reactivity and cross-protection between different HPV types.

6. Clinical Vaccine Development

At present, there is no HPV-specific vaccine on the market; however, several clinical trials evaluating the safety and immunogenicity of HPV 6, 11, and 16 vaccines are ongoing or have already been completed. Only a few of them have so far been published in peer-reviewed journals or book chapters and the readers are referred to meeting reports or the websites of the companies sponsoring these trials.[236,262-284]

Independent development of prophylactic vaccines against HPV 16 and HPV 18 infection have been driven by the pharmaceutical industries in collaboration with researchers from academic institutions. Some details of the study designs and preliminary results have been disclosed,[236,269,270,275,279,285] and readers are referred to the company websites for updated information.[286,287] Based upon results of a dose escalation trial in young, healthy individuals using HPV 16 VLPs[278] and upon data from a cohort of women from Guanacaste/Costa Rica[250,288] the US National Cancer Institute is preparing a large prophylactic trial in Costa Rica to prevent persistent HPV infection and CIN.[235]

The existing data from the initial prophylactic trials demonstrate that: (i) application of VLPs into humans is well tolerated; and (ii) VLPs are highly immunogenic at relatively low doses (3×10–$50\mu g$) and, even in the absence of adjuvants and induce high-titer neutralizing antibodies.[278] Data about protection against infection have just been obtained (Koutsky L, personal communication) and, reports about clinical efficacy are being awaited.

Therapeutic trials are being conducted in patients with late-stage cervical cancer or high-grade intraepithelial neoplasias (i.e. CIN or other HPV-associated genital lesions, such as anal intraepithelial neoplasia [AIN] and vulval intraepithelial neoplasia [VIN]). Generally, all therapeutic vaccines were reasonably well tolerated and showed an immune response in some patients. For more detailed information about some of the early therapeutic trials (see Gissmann et al.[225] and Tindle[289]). There were no remarkable clinical responses observed in trials with cancer patients.[262-264,268] Preliminary information on the efficacy of these therapeutic vaccines has been published for some of the therapeutic trials of patients with premalignant HPV-induced lesions. Clin-

ical response was reported in some of the vaccine recipients: (i) in HLA-A2-positive patients with either HPV 16-positive high-grade CIN or VIN treated with an HPV 16 E7-derived peptide vaccine;[271] (ii) in patients with high-grade AIN after treatment with an Hsp-HPV 16 E7 fusion protein;[277,284] and (iii) in patients with VIN 3 immunized with HPV 16 and 18 E6/E7 recombinant vaccinia.[280] These very promising results can be taken as the first hint that the concept of an HPV-specific immune therapy may actually be successful. Everyone in the field is looking forward to learning about more comprehensive data after the completion of larger trials.

7. Conclusion

Cervical cancer is reasonably well controlled in the western world but represents the second most frequent malignancy in women in less developed countries. As initial clinical vaccine trials yield promising results, an effort has to be made to make such vaccines available to less affluent societies as well. This requires not only political action but also the creativity of the researchers in the field to offer solutions for a more economical production, higher stability, and safer application of the vaccine. If successful and used on a large scale, HPV-specific vaccination could prevent millions of cancer deaths. Whereas cancer prevention by vaccination has already been successfully demonstrated (hepatocellular carcinoma related to infection by the hepatitis B virus[290]), treatment of persistent HPV infection (with or without clinical symptoms) by therapeutic vaccination would be the first example of successful cancer control by immune therapy.

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