

VACCINES AGAINST HUMAN PAPILLOMAVIRUSES – A MAJOR BREAKTHROUGH IN CANCER PREVENTION

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SUMMARY

Carcinoma of the cervix (CaCer) is the second most frequent malignancy in women on a global scale. Epidemiological studies carried out at the beginning of the second half of the 20th century showed that CaCer was of infectious nature and that its agent was transmitted by sexual intercourse. For some 15 years, herpes simplex virus type 2 (HSV2), the genital herpes virus, was suspected to be the etiological agent. This hypothesis was disproved just in the time when the first convincing evidence that the agents of the disease were human papillomaviruses (HPVs) was produced. Copious new findings obtained during the 1980's and 1990's unequivocally confirmed that HPVs were the causative agents. The most dangerous among the over 100 HPV types are types 16 and 18, which together account for over 70% of CaCer cases and very likely also for most of the other malignancies of the anogenital region and the oropharynx. Extensive research of the HPV biology and immunology enabled the development of vaccines based on the s.c. virus-like particles (VLP) prepared by genetic engineering. At present, there is one HPV vaccine on the market; it contains, besides types 16 and 18, also types 6 and 11, the causative agents of certain benign tumours of the genital area and of the larynx. A new vaccine, comprising types 16 and 18 only, the product of another firm, is to appear on the market soon. Both vaccines have already been tested in extensive clinical trials. They are nearly 100% effective, only very weakly reactogenic and they undoubtedly belong among the most perfect vaccines ever produced. The darker side of the anti-HPV vaccines is their high price, the fact that the highest benefits they bring will only become evident in 20 or 30 years, and that they do not afford protection against all oncogenic HPVs. It is therefore imperative that organized cytological screening be continued: it is destined to remain the main instrument of CaCer prevention for several decades. With all probability also other types of vaccine are under development, viz. VLP-based vaccines, whose range of applicability will be wider than that of the present preventive vaccines, as well as vaccines that will, hopefully, be able to inhibit already progressing infection or will be utilizable in CaCer immunotherapy.

Key words: carcinoma of the cervix, papillomavirus, HPV vaccines

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INTRODUCTION

Carcinoma of the cervix (CaCer) is the second most frequent malignancy in women on a global scale. Some half a million new CaCer cases appear in women per year. About 60% of them die of the disease later. Its aetiology was given extraordinary attention in the second half of the previous century. Two circumstances occasioned this. First, the great expectations that the disease would be eradicated, which were aroused by the introduction of cytological diagnosis of precancerous lesions in the 1940s, were not fulfilled. Second, CaCer was found to be extensively prevalent in the developing countries. The highest number of women that die there of malignancy are CaCer patients.

CARCINOMA OF THE CERVIX HAS FEATURES OF AN INFECTIOUS DISEASE

Research in CaCer, in which the most outstanding epidemiologists of the time engaged at the beginning of the second half of the 20th century, soon brought indubitable evidence that the development of the disease was associated with the style of sexual life. The highest risk factors appeared to be a high number of partners and early start of sexual activity (1,2). Other risk factors were also recognized, viz. the socioeconomic status, a high number of births, a high divorce rate, etc., but in statistical analyses they oozed out or lost significance. Another important factor appeared to be the so-called male factor (3,4):

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the second wives of men whose previous spouse had suffered from CaCer were under an enhanced risk of developing the malignancy, and so were monogamous wives of promiscuous men. Later on, smoking was recognized as a potent risk factor. In spite of being closely associated with the sexual life style, smoking apparently also functions as an independent risk factor (5).

In short, the picture emanating from the results obtained mainly in the late 1950s and early 1960s provided unequivocal evidence that CaCer had the character of an infectious disease and that the causative infectious agent was transmitted by sexual intercourse. Consequently, extensive hunting for the etiological agent began.

HERPES SIMPLEX TYPE 2 AND CARCINOMA OF THE CERVIX

In spite of the efforts of many laboratories, which tested the familiar sexually transmissible agents for CaCer induction, nothing was found that would cast even a trace of suspicion on any of them for a long time. A dramatic change came by the end of the 1960s, when two research groups in the USA, independently of each other and almost simultaneously, reported that patients with CaCer had antibodies to herpes simplex virus type 2 (HSV2) much more frequently than normal women. A short time before this it had been found that the HSV viruses, heretofore held to form a homogeneous group, actually consisted of two virus types: one of them, predominantly transmitted with saliva, was designated “oral” or type 1 (HSV1), the other, mainly spreading through sexual intercourse, “genital” or type 2 (HSV2) (6,7). HSV1 and HSV2 differ mutually by a number of biological characteristics and somewhat also by their antigenic structure, which enables their serological differentiation. The initial observation of a more frequent presence of anti-HSV2 antibodies in CaCer patients than in normal (or otherwise affected) women was soon confirmed in tens of further studies [for reviews see (8,9)] including a study of ours performed among the Czech population (10). Other observations continued to appear and suggested HSV2 was a very probable candidate for the causative agent of CaCer. In particular, the virus was shown to have an oncogenic potential for rodents (11,12), but some other experimental results were also strongly suggestive. The virus experimentally induced dysplastic changes on the cervix in monkeys (13) and several laboratories succeeded in detecting HSV2-specific macromolecules, i.e. viral DNA (14,15) or virus-specific proteins (16-18) in cells taken from CaCer. Furthermore, the prevalence of CaCer and the spread of the virus displayed similar epidemiological characteristics. The hypothesis of an etiological relation of HSV2 to CaCer was moreover strongly, though indirectly, supported by the accumulating evidence that another herpetic virus, viz. the Epstein-Barr virus (EBV), played a role in the pathogenesis of certain other human tumours (Burkitt’s lymphoma, nasopharyngeal carcinoma, possibly others) and that, under natural conditions, animals of different species developed malignant tumours induced by their species-specific herpetic viruses. By the end of the 1970’s it was nearly generally accepted that HSV2 was the etiological agent of CaCer and researchers began to think of the development of a preventive vaccine.

In spite of all the evidence for an etiological HSV2–CaCer relationship, critical scrutiny of the sum of the findings revealed certain weak points. The main were not complete reliability of the serological tests used for anti-HSV2 antibody detection and the fact that all of the serological studies performed were retrospective, i.e. case-control studies, and thus there was no real certainty that HSV2 infection preceded the disease. Furthermore, HSV2-specific macromolecules were not invariably demonstrable in human CaCer. The virus non-virion antigens that used to be found differed from laboratory to laboratory, antibodies against them were not regularly detectable in patients, and even by some of their other characteristics they did not completely resemble the virus proteins routinely detectable in the cells of thoroughly investigated tumours induced by animal oncogenic viruses. The findings evidently did not fulfil the criteria for confirming an etiological relationship between virus and tumour as were formulated by us later on (19). However, the doubts were more or less dispelled by an explanation based on some critical analyses coming from laboratories which in the early 1980’s played a foremost role in the research into the role of viruses in CaCer pathogenesis (20, 21). According to these authors, HSV2 did not behave according to the “hit-and-stay” principle, as did other tumour viruses, but in the “hit-and-run” manner, as did chemical carcinogens. In other words, the virus was needed for cell transformation but not for the maintenance of the transformation state. This hypothesis, in every way a novelty, was compatible with all the findings known by then and offered an explanation for those which aroused certain doubts. Its weightiness increased upon the demonstration of a mutagenic ability in one virus product (22).

In order to supply, as it seemed at that time, the last missing piece of evidence that HSV2 was indeed the decisive etiological factor in the development of cervical neoplasias, including CaCer, an extensive prospective study was undertaken in Prague; it was carried out by our team in cooperation with Jiří Kaňka and his co-workers (23). Over 10,000 initially normal women were followed up for six years in the late 1970s and early 1980s. At the enrolment, all of them were examined cytologically and colposcopically and serum samples, anamnestic data and detailed information about their habits, especially the style of their sexual life, were obtained from all of them. Our reasoning was as follows: if HSV2 was the causative agent of CaCer, then the development of CaCer and precancerous lesions on the cervix would have to be significantly higher in those women who had been infected by this virus prior to the follow-up than in those who had not. Under our arrangement we had a very high number of control women and were thus able to match those who developed CaCer in the course of the study (advanced precancerosis in most cases, micro invasive CaCer rather exceptionally) with controls who concurred with the patients in the most important risk and protective factors but remained normal until the end of the observation period. Such matching had not been possible in any of the previous studies. We also developed a new, highly reliable test for differentiating anti-HSV1 from anti-HSV2 antibodies (24). When, upon completion of the work in the field, we tested a large number of selected sera, the result was quite surprising: women who had developed cervical neoplasia (i.e. precancerous lesions and in a few instances microinvasive CaCer) in the course of the study had anti-HSV2 antibodies as frequently as those who had not (25). The implication was as clear: HSV2 was not the causative agent of CaCer.

Similar results to ours, i.e. no evidence of etiological relationship between HSV2 and CaCer, were subsequently obtained by two other groups (26, 27).

THE BIRTH OF THE HYPOTHESIS OF A RELATIONSHIP BETWEEN HUMAN PAPILLOMAVIRUSES (HPVS) AND CARCINOMA OF THE CERVIX

By the time we had completed our study there appeared first reports suggesting that the agents involved in CaCer pathogenesis were HPVs. These viruses had long been known as inducers of benign human tumours, in particular warts, and few virologists suspected them of being involved in the development of any human malignancies. Among this very small minority, a dominant figure was the German virologist Harald zur Hausen. He was convinced that actually HPVs played a key role in the development of CaCer and undertook to verify his hypothesis. Nevertheless, he conceded the participation of HSV2 as an important co-carcinogen. The first reports from his laboratory that considerably influenced the direction of further research came out in 1983 and 1984 (28, 29). They provided evidence for the presence of DNA of two new HPV types, designated 16 and 18, in CaCer biopsies. Since they appeared at the same time as the role of HSV2 in CaCer pathogenesis was disproved, they aroused extraordinary interest. The new findings were soon confirmed at other laboratories and in the following years studies on the relationship of HPV to CaCer started on a global scale. Were the HPVs the causal agents, or were they merely passenger viruses that had an affinity to the neoplastically altered tissue? In view of the biological characteristics of HPVs, the investigations were far more difficult than the previous work with herpetic viruses, because HPVs cannot be cultivated on any of the usual types of tissue culture. Owing to this, molecular biology methods became the main providers of new findings. Interpretation of the results was impeded by it

being impossible to apply Koch's postulates – which are normally used for clarifying causal relations in medicine since the end of the 19th century – in the case of viruses suspected of participation in the development of human malignancies. In order to reliably confirm a causal relationship between HPVs and CaCer, it was necessary to collect molecular-biological, immunological and epidemiological data and make sure that they all harmonized. Thanks to the efforts of tens of virology laboratories such evidence was eventually obtained. It is summarized in Table 1. Probably the most important items are the last two under the heading "Epidemiology". The studies performed have proven that HPV infection precedes the development of CaCer [helpful in this respect were also serological tests for HPV antibodies (30) and examination of cytological smears for HPV presence (31) of the materials collected in the above-mentioned Prague prospective study] and that vaccination against the infection (see below) prevents the development of neoplasia of the cervix. To use the words of the learned medieval Dominican, Thomas of Aquinas, "*Sublata causa, tollitur effectus*".

By the present, the role of HPVs in CaCer pathogenesis has been firmly established. As mentioned above, the most important are types 16 and 18. Together they are responsible for about 70% of CaCer cases. Other types are found rarely (Fig. 1). However, it is evident that HPVs alone do not induce the disease. Thus, these viruses are a *necessary* but not a *sufficient* condition. The evidence for this is that only a very small minority of those infected develop CaCer. For CaCer to develop, the participation of certain other factors is necessary, the most important of which seem to be the carcinogens present in tobacco smoke and a variety of infectious agents that produce inflammatory lesions in the cervix. Experimental studies have provided some of the evidence. Viral oncogenes alone (see below) are able to immortalize *in vitro*-cultivated cells but not to provide them with an oncogenic potential. To gain such a potential, the cell must receive a further activated oncogene, e.g. a mutated *ras* oncogene.

Table 1. Evidence of etiological association between HPVs and cervical cancer¹⁾

Direct	
Epidemiological	
(i)	infections with HPV are more frequent in patients than in matched healthy subjects
(ii)	the spread of HPV and the incidence of cervical neoplasia have similar epidemiological characteristics
(iii)	HPV infection precedes the development of cervical neoplasia
(iv)	HPV vaccine prevents the development of cervical neoplasia
Immunological	
(i)	patients with cervical neoplasia possess much more frequently antibodies to viral oncoproteins (E6 and E7) than healthy HPV-infected control subjects
(ii)	there is a relationship between the levels of antibodies against viral oncoproteins and the clinical condition and prognosis of the patients
Molecular biological	
(i)	tumour cells regularly contain HPV specific macromolecules (DNA, RNA and proteins)
Indirect	
(i)	genes for HPV oncoproteins (E6 and E7) are capable of immortalizing mammalian cells cultivated <i>in vitro</i>
(ii)	inactivation of HPV oncogenes in transformed cells results in the loss of malignant phenotype
(iii)	animal papillomaviruses induce tumours in the respective animal species

¹⁾ Based on methodology described and substantiated in Vonka V, Philos Trans R Soc Lond B Biol Sci. 2000, 355, 1831 (19)

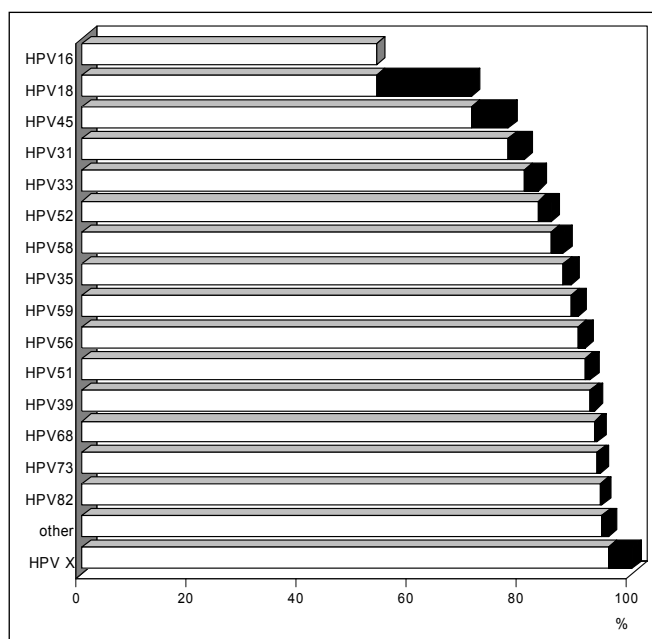


Fig. 1. Percentages of cervical cancer cases linked to most frequent HPV types worldwide. According to Munoz N et al, *IJC* 2004, 111, 278 (39).

THE BIOLOGY AND EPIDEMIOLOGY OF HPVS

Since the year 2000 the papillomaviruses (PVs) are classified as a separate family, the *Papillomaviridae*. They are small, non-enveloped DNA viruses 55 nm in size. The PV genome, a covalently closed, double-stranded DNA molecule comprising about 8000 base pairs (Fig. 2), may be divided into three regions: region E (early), which codes for as many as seven early proteins that are not included in the virion structure; region L, which carries the genetic information for two envelope proteins, L1 and L2, and the non-coding LCR region (long control region), which includes sequences that regulate the life cycle of the virus and interact with cellular and viral proteins. All of the coding sequences are situated on one strand of the virus DNA. Proteins E1 and E2

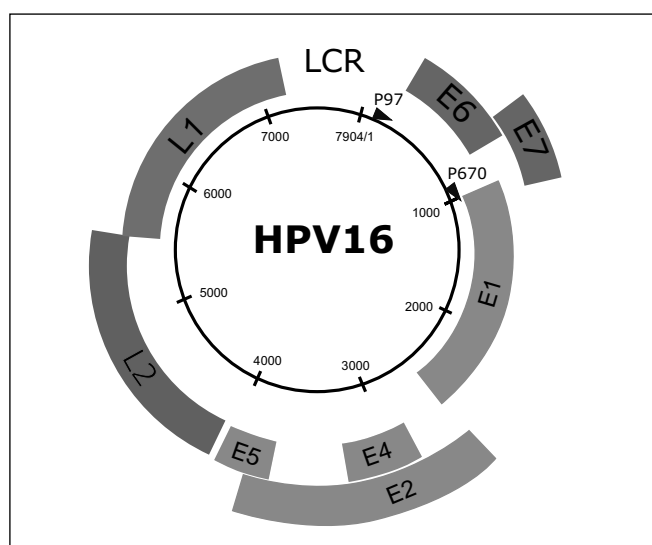


Fig. 2. Organization of HPV16 genom.

mainly operate at the early stage of virus growth, protein E4 during virus maturation. For carcinoma development, the most important are the E6 and E7 proteins of the high-risk HPV types (see below). Interest centred upon them when it transpired that their properties were similar to those of the oncoproteins of certain other oncogenic DNA viruses, such as the polyomaviruses and adenoviruses. They bind to and inactivate the products of the two most important tumour suppressor genes: E6 inactivates p53 and E7 inactivates pRb105 and related proteins. In this way and by interacting with some other cell proteins they create conditions for uncontrolled cell growth.

Papillomaviruses have been isolated from quite a number of the higher vertebrates including man. They are not transmissible between species. They have been classified on the basis of species specificity and similarity of their genomes. The individual types differ by more than 10% of the L1 gene sequence. At present, at least a partial genetic sequence is known for over 300 PV types; of these, about 120 can infect man. Papillomaviruses are strictly tissue-specific: they only infect epithelial cells of the skin and the mucosa. Susceptible to PV infection are immature cells of the basal layer of the epithelium, whereto the viruses penetrate after microscopic injuries. The interface between the squamous and the cylindrical epithelium on the cervix, the epiglottis and in the anal region is particularly susceptible to PV infection. The PV replication cycle is closely associated with the differentiation of the cell infected: viral DNA in the episomal form replicates under E1 and E2 protein control in the nuclei of basal cells and their progeny, so-called transient cells, with the help of the replication machinery of the host cells. The simultaneous expression of other early proteins (E6 and E7) influences the division and vertical expansion of the infected cells and puts off their transition from the proliferative to the differentiation stage. Virus capsid proteins are expressed in the upper layers of the differentiating epithelium, while complete infectious virus particles develop in terminally differentiated keratinocytes. The virions are released as the surface cells desquamate and they can then become source of infection for other subjects.

Papillomaviruses are classified as skin and mucosal according to their tissue specificity, and as low-risk (LR) or high-risk (HR) types according to their ability to induce malignancy. The human genital tract may be infected by about 40 HPV types. At present, 15 of them are qualified as oncogenic (HR) types, and three others as potentially oncogenic. The rest are LR types.

Genital HPVs are the most frequent sexually transmitted virus agents. During lifetime, as many as 80% of sexually active women and men encounter HPV infection (32). The infectious potential of HPV is comparable to that of sexually transmissible bacterial pathogens. The other virus pathogens transmitted in the same manner (HIV, HSV2) are much less infectious (33). Most often, HPV infection occurs soon after the onset of sexual life. The cumulative incidence of HPV infection among girls previously HPV-DNA-negative is 40–60% after 3 years (34, 35). Collins et al. have furthermore shown that the average span between the first sexual intercourse and first detection of HPV DNA is 3 months (36). In most young women the infection is asymptomatic, can only be detected by molecular biology methods (HPV DNA detection) and is usually transient. In women above 30 years of age infection by genital HPV types is much less frequent, but tends to persist which involves the risk of intraepithelial lesion development.

Genital warts are typical clinical manifestation of infection by a LR HPV types; in over 90% HPV type 6 or 11 are involved. The same PV types account for the development of recurrent laryngeal papillomatosis. Only exceptionally can LR HPV types be found in lesions that have progressed into malignancy. Persistent HPV infections accompanied by ongoing virus replication and a high virus load may induce intraepithelial lesions of growing seriousness (37, 38). In current classification (Bethesda) these lesions are distinguished into low-grade squamous intraepithelial lesions, LSIL (in previous histological classification denoted CIN1 or the less serious form of CIN2) and high-grade squamous intraepithelial lesions, HSIL (previously denoted advanced CIN2 or CIN3), among which carcinoma *in situ* is also included. The final stage of the pathological process is CaCer. As the seriousness of the disease grows, the spectrum of HPV types diminishes, until CaCer cells only contain the oncogenic HPV types, predominantly HPV16, which accounts for 53.5% of cases on the global scale, followed by HPV18 which is involved in 17% of cases (39). Whereas the incubation period for the development of genital warts may last in the order of months, the period between infection and the appearance of the carcinoma usually takes some 20 years or more, with only exceptionally clinically manifest carcinoma appearing after a significantly shorter time.

Since the pathological stages that precede CaCer are known, and so are the infectious causative agents, it is possible to substantially reduce its incidence by preventive programs. At present, CaCer prevention is mainly provided at a secondary level, viz. by cytological screening that detects abnormal cells in cervical swabs. The implementation of organized screening, under which at least 80% of the female population within a certain age span were covered and accuracy of the work was ensured at all levels (cytological laboratory, gynaecology, therapy), reduced in Finland CaCer incidence from 14.9 to 3.3 per 100,000 women between the 1960s and 2005 (<http://www.cancerregistry.fi/stats/eng/veng0006i0.html>). The average CaCer incidence in the European Union countries was 12.2 per 100,000 women in 2002, while in the Czech Republic it has been oscillating around 20/100,000 through many years (<http://www.uzis.cz/>) (40).

DEVELOPMENT AND PROPERTIES OF PREVENTIVE ANTI-HPV VACCINES

Ever since the etiological relationship between HPV infection and CaCer development was established, the endeavour of researchers involved in this particular field of study has aimed at the development of a vaccine that would prevent the entrance of HPV into susceptible cells. As stated above, the study of HPVs is not easy because of their biological characteristics and its progress is fully dependent on molecular biology methodology. Similar procedures had to be applied in the development of the vaccines. The first step in the development and preparation of preventive vaccines was the recognition that the major virus capsid protein, L1, if produced in big enough amounts in recombinant systems is able to self-assemble in not only pentamers structures, designated as capsomers, but also in so called virus-like particles (VLP, capsids). The VLPs are indistinguishable from infectious virions morphologically, but they do not contain virus DNA and therefore can neither multiply in susceptible cells nor transform

them (41–44). Antigenic epitopes on their surface are able to induce production of neutralizing antibody.

After the success of experimental studies on animals, the first clinical tests were initiated in the middle of the 1990s. The HPV types chosen for the preparation of experimental vaccine were HPV16 and 18, the most prevalent oncogenic types worldwide, responsible for over 70% of CaCer cases (see above). In the course of years vaccines of this kind proved to be safe, immunogenic, capable of preventing both persistent infection and development of clinically evident lesions caused by vaccinal HPV types. At present, the first vaccine is already commercially available, it was developed by Merck and Co. under the commercial name Gardasil; in a part of the European Union, including the Czech Republic, it is available as Silgard. A second vaccine Cervarix, developed by GlaxoSmithKline (GSK) has been registered in Australia in spring this year and will probably be commercially available in Europe by the end of 2007. Both vaccines are based on highly purified VLPs prepared by recombinant technologies, but they differ in several respects. Difference one is in their composition: Gardasil contains, besides type 16 and 18 VLPs, also LR VLP type HPV6 and 11; second, it is prepared in a yeast expression system, while Cervarix is produced using recombinant baculoviruses on insect cells. Third, VLPs in Cervarix are adsorbed on ASO4, an adjuvant developed by GSK, which contains in addition to amorphous aluminium salts 3-deacylated monophosphoryl lipid A, which is a detoxified lipopolysaccharide of *Salmonella minnesota*. The ASO4 adjuvant has proved to be of value in an anti-type B hepatitis vaccine. A three-dose immunization scheme is used with either vaccine.

Randomized, double-blind clinical studies with either vaccine were carried out in many countries, including the Czech Republic. Both vaccines induced high levels of antibodies against vaccinal HPV types. The highest antibody levels were repeatedly detected 7 months after the 1st dose (i.e. 1 month after the 3rd dose). Thereafter the antibody titres decreased, but as from month 18 they kept stable for 5.5 years (so far the longest follow-up period reported) (45). The vaccination effectiveness against persistent infection, defined as repeated findings of identical type HPV DNA in smears collected at 6-month intervals, was 100% for the Cervarix (GSK vaccine) and 96% for the Gardasil (Merck&Co. vaccine). Women who showed no signs of previous infection at entry in the study and were then properly vaccinated with either vaccine, had no signs of cervical intraepithelial lesions associated with the vaccine HPV types throughout the observation period (46, 47). The administration of the tetravalent vaccine also prevented the development of external genital lesions.

Table 2 presents data obtained at the third stage of clinical studies with the Gardasil vaccine, the point of interest there being the development of cervical intraepithelial neoplasia, stage 2 or worse (CIN2+), and *in situ* adenocarcinoma. Women who had been seronegative on day 0 and HPV DNA-negative throughout the immunization protocol, as well as women who had had specific antibodies at enrolment in the study (those who had been infected but had cleared the virus), were 100% protected from the disease. On the other hand, the studies showed that the vaccination had only a weak effect on lesion development in incidentally infected women (HPV DNA+, seronegative women), whereas women experiencing persistent infection (HPV DNA+, seropositive) were not protected at all. The results of clinical studies performed with

Table 2. Gardasil efficacy against vaccine HPV related CIN2+ in women with various virological findings at enrolment in the study

Vaccine		Control		Observed efficacy (%)
Number	Number of cases1)	Number	Number of cases ¹⁾	
Naive population (Day 1 PCR–/Serologically–; Month 7 PCR–)				
8487	0	8,460	53	100
Cleared infection (Day 1 PCR–/Serologically+)				
853	0	910	0	100
Early infection (Day 1 PCR+/Serologically–)				
661	42	626	56	31.2
Chronic infection (Day 1 PCR+/Serologically+)				
568	75	580	69	<0.0

¹⁾ Females with histologically proved cervical intraepithelial neoplasia grade 2 or worse finding associated with vaccinal HPV types

the divalent Cervarix vaccine in the group of women infected with vaccinal HPV types at enrolment in Costa Rica imply that the vaccination had no virus-clearing effect (Fig. 3) (48).

In view of the almost generally shared conviction that mainly preadolescent girls should be vaccinated, the results of studies that tested the ability of vaccines to induce an antibody response in children are of particular importance. Studies with both vaccines performed showed that the antibody levels were significantly higher in girls 10–14 years old than in young women aged 15–25. It may thus be expected that the immunization of girls would afford long-term protection both against HPV infection and the development of HPV-associated lesions (49, 50).

The side effects after the administration of either HPV vaccine were minimal. Local reactions (pain, swelling, redness) were somewhat more frequent in the vaccinated group than in a group given the adjuvant alone, but the difference was not statistically

significant. Comparison with a group given only saline as placebo indicated that the majority of post-vaccination reactions could be attributed to the adjuvant based on aluminium salts.

Phase III and IV studies, in which tens of thousands of women but also children and men are followed, are being continued. They focus on long-term safety, on vaccination-induced immunity and on protection against persistent infection and the development of lesions induced by the HPV types contained in the vaccine. Another objective is to ascertain whether the vaccines can also protect against HPV types phylogenetically related with the vaccine types. The first results suggest that this might be so with either vaccine, at least as regards types HPV31 and HPV45 (45).

VACCINATION POLICY

It is an indisputable fact that the present anti-HPV vaccines confer a high degree of protection against infection by the most dangerous HPV types, can protect from the development of neoplastic lesions induced by these viruses and that are only very weakly reactogenic. They belong among the most perfect vaccines ever produced. Nevertheless, there is no general agreement as to how the preventive vaccination should be administered. The authors of this paper side with those who recommend state-financed overall mandatory vaccination of preadolescent girls. It would be optimal to vaccinate each year all girls who in that calendar year have or will have reached a certain age, e.g. 12 or 13 years. If vaccination is to be effective, it must precede infection. We insistently recommend that elder girls and women be vaccinated as well. Their vaccination should be voluntary and should be paid by the vaccinees, fully or partially within their health insurance fees.

Apart from their advantages, viz. a high effectiveness and negligible reactogenicity, the vaccines also have some weaker points. These include a high price, the fact that its main effect, the reduction of CaCer incidence, can only become evident 20 or 30 years after the vaccination, and its inability to protect against all the HPV types that can induce malignant growth on the cervix (see Fig. 1). It follows that organized cytological screening should continue on an unreduced scale in both vaccinated and unvaccinated women. Health education must keep a close watch

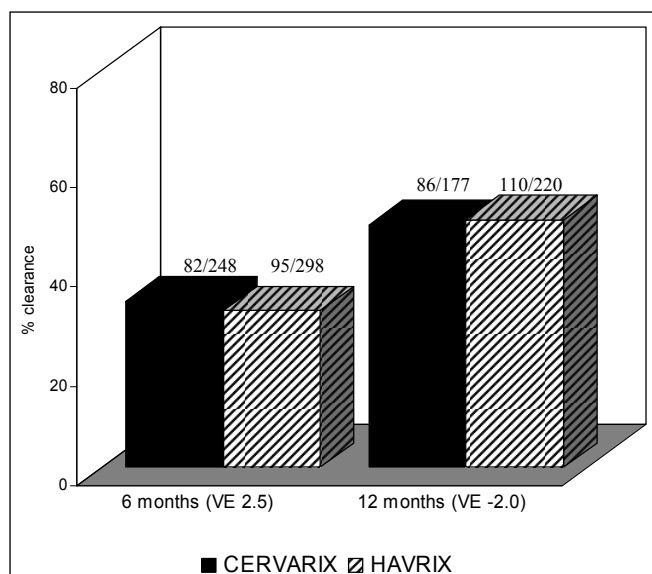


Fig. 3. Clearance of HPV infection in women infected at enrollment with HPV16 or 18 vaccinated by Cervarix (HPV16/18 divalent vaccine) and Havrix (control group vaccinated by hepatitis A vaccine) 6 and 12 months after vaccination. According to Hildesheim A et al, JAMA 2007, 298, 743 (48).

on the facts that vaccinated women do not build up a false feeling of safety and of being completely protected against CaCer or even against all sexually transmissible diseases. The opponents of overall vaccination often argue that its costs are high and its effect on CaCer incidence can become evident only after a long time, while they overlook the fact that a reduction of the incidence of precancerous cervical lesions will come much sooner. Their treatment and possibly long-term follow-up represent no small expenses. Present-day estimates indicate that vaccination with the contemporary vaccines will lower the incidence of mild lesions, the majority of which are caused by the so-called low-risk HPV types, by some 20%, and the incidence of severe lesions, predominantly induced by the vaccine types, by 60%.

Another reason for wide-scale vaccination is that continuing research has been gradually discovering the involvement of HPVs also in the development of some other human malignancies. According to present-day knowledge, some 40% of carcinomas of the vagina and the vulva, 50% of carcinomas of the penis, 90% of carcinomas of the anus, and about 25% of carcinomas of the head and neck (mainly the oropharynx) are also induced by HPVs, with the vaccine type 16 being the predominant agent (51, 52). It can thus be expected that systematic vaccination will lead to a gradual reduction of the incidence of all of these carcinomas. Moreover, as has already been pointed out, a very great majority of genital warts and laryngeal papillomatosis cases are induced by HPV types 6 and 11, whose VLPs are constituents of a vaccine that is on the market already at present. Although these diseases are not malignant, they are most unpleasant and difficult to cure. In their case, the beneficial preventive effect of vaccination should appear substantially earlier than a reduction of carcinoma incidence. Expectedly, systematic vaccination of pre-adolescent girls would lead to complete eradication of child laryngeal papillomatosis in the course of two or three decades.

HAS THE DEVELOPMENT OF HPV VACCINES BEEN COMPLETED?

In the opinion of the authors of this paper, it has not. The contemporary vaccines can reduce the future incidence of CaCer by 70%, possibly – if the findings on cross immunity with several other high-risk HPV types are confirmed – by 80%. It is highly probable that vaccines are already being developed which, thanks to the inclusion of some additional HPV types, will have a broader range of effect, i.e. they will be able to reduce CaCer incidence by 90% or more. It is also possible that the continuing research in HPV immunology will lead to the development of a mixed vaccine, a sort of a “cocktail” of PV antigens, which might be able to prevent infection by any of the high-risk HPVs. No doubt a different type of HPV vaccine is also under development, viz. vaccines that will be able to provoke an immune response capable of counteracting established HPV infection. As pointed out above, existing HPV infection represents a situation upon which the contemporary vaccines, directed against the main structural protein of the virus, have no effect. Such “secondary prophylactic vaccines” would have to be based on non-structural HPV early proteins, most likely proteins E1 and E2. These proteins have their say at an early stage of replication of the virus. A recent success of such a vaccine in the treatment of lesions caused by a rabbit PV

is an encouragement for those endeavouring to prepare a similar vaccine for humans (53).

One of the dominant trends in contemporary oncology research is the development of therapeutic vaccines, whose use should supplement the contemporary therapeutic procedures employed in oncology. Although the successes achieved in the immunological treatment of malignancy have not been dramatic so far, the progress in knowledge of the immune responses of the organism to malignant tumours and of the mechanisms whereby they could be enhanced, as well as the rapidly accumulating new findings in tumour biology, are a big promise for the future. Obviously, virus-induced tumours, primarily those induced by PVs, are also in the centre of interest. There are several reasons for this. Even if the entire population were to be vaccinated with preparations that would ensure protection against infection by any of the oncogenic HPVs, CaCer would continue to develop for several decades, since tens of millions of women are in the incubation period. From the immunological point of view, virus-induced tumours have the advantage over other neoplasms that they carry large, well-defined virus proteins, which represent attractive targets for immunotherapy. However, immunity that would result in rejection of a tumour would obviously be of a different type than that inducible by the prophylactic vaccines of today. Whereas in the case of the prophylactic vaccines the active agent are antibodies against the virus capsid antigens, which neutralize the virus present on the mucous membrane surface, tumour rejection requires cytotoxic T lymphocytes specifically activated against the E6 and E7 virus oncoproteins. These oncoproteins are constitutively formed in tumour cells and are functionally responsible for the development and persistence of the malignant phenotype. They represent real tumour antigens. At present, intensive work aiming to develop therapeutic vaccines against HPV-induced tumours is under way. Some of the experimental vaccines based on genetic engineering procedures are undergoing clinical tests. Although immune responses have been demonstrated by *in vitro* tests, clinical responses were not particularly distinctive. Hence the centre of gravity of the research is in experimental animal models for the time being. DNA vaccines, recombinant viruses, peptide vaccines, various types of recombinant proteins, as well as vaccines prepared from tumour cells genetically modified so as to produce cytokines, are all at a testing stage. Tumour cells genetically modified as just indicated are capable of substantially raising the immune response to tumour antigens. The research now under way also includes investigation of the mechanisms whereby tumour cells escape immune surveillance and, in particular, aims at recognition of the processes whereby the tumour, in its microenvironment, protects itself from the immune counterattacks of the organism, and at finding ways of annulling these tumour-protective countermeasures. If the present optimistic assumptions are confirmed, immunotherapy could within five years' time begin to play an important role in the treatment of HPV-induced tumours.

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