Editorial Articles

Anti human papillomavirus vaccine: The checkmate to human papillomavirus?

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Summary

Cervical cancer is the second most frequently found neoplasia in women worldwide. At least 95% of cervical cancers contain viral DNA which, in 80% of cases, belongs to one of the human papillomavirus (HPV) viral types at high oncogenic risk: 16, 18, 31 and 45. HPV is, at this point, considered the first "necessary cause" of cervical cancer, against which primary prevention with a reduction in the risk of infection can be carried out. Numerous molecular biological studies have been conducted to identify the biological markers of this infection and to refine an effective and well tolerated vaccine capable of preventing HPV infection as well as possibly treating those cases in which the infection has already caused an HPV-related disease of the genital tract. In the near future, the real primary prevention of this disease will be conducted, similar to what occurred for Hepatitis B, using immunoprophylaxis with an anti-cancer vaccine.

Key words: Human papillomavirus; Vaccine; Clinical trial.

Introduction

The first vaccine is 205 years old. In 1798, Edward Jenner discovered, all entirely by chance, prophylactic immunization against smallpox by inoculating humans with the purulent material taken from the pustules of a disease in cattle, called vaccinia (cowpox), and thus developing the first live vaccine. The term vaccine, therefore, derives from the vaccinia virus of the family of *Poxvirus*, a virus of a different species containing an antigen common to the human virus.

Since that time, the different vaccines have consisted of one of the following substrates: 1) inactivated dead whole microorganisms; 2) attenuated live microorganisms; 3) subunits of microorganisms (subunit vaccines): proteins (bacterial toxoids = inactivated toxins), polysaccharides or DNA (DNA vaccines). Antigens can be obtained through both biochemical and genetic recombination techniques [1].

Thus, for more than 200 years, viral diseases have been fought and "controlled" exclusively through immunization; it is impossible not to recall the results obtained through vaccination against diseases such as smallpox and poliomyelitis.

The term vaccine is, however, beginning to take on significance also in the field of oncology. Anti-cancer vaccines are not fundamentally different from the classical ones in that their objective is to render the host immune system "reactive" against the oncogenic antigens.

Human Papillomavirus Diseases

One of the most important discoveries in the etiology of human neoplasia has been the relationship between HPV infection and human cervical neoplasia.

In terms of public health, this discovery is just as important as the relationship between cigarette smoke and lung cancer or between chronic HBV (Hepatitis B virus) or HCV infection and liver cancer.

HPVs infect the skin and mucosa, causing both benign and malignant hyperproliferative lesions [2]. Condylomata acuminata lesions, caused by low-risk oncogenous viral types 6 and 11 in 95% of cases, are lesions that, although they do not tend towards malignant transformation, generally provoke high psycho-social morbidity [3].

In the United States, it is estimated that there are approximately 1,000,000 new cases of low-grade squa-

mous intraepithelial lesions (SIL) every year, around 300,000 new cases of high-grade SIL, and around 10 million new cases of cervical infection due to HPV not associated with cytologic abnormalities. At least 95% of cervical cancers contain viral DNA which, in 80% of cases, contains one of the following types of high risk oncogenes: 16, 18, 31, and 45 [4].

In the 1990s, epidemiological studies supported by molecular technology clarified the relationship between HPV infection and human cervical neoplasia. As far as is presently known, the causal role of persistent human papillomavirus infection in the development of cervical cancer and its precursors is now sufficiently documented beyond any reasonable doubt.

Longitudinal studies demonstrate that viral infection precedes the development of all high-grade squamous lesions and that the distribution of viral genotypes in high-grade SIL is comparable to that observed in cervical cancer. HPV infection is an important biomarker associated with the development of high-grade squamous lesions [4]. The virus has been proposed as the first ever identified "necessary cause" of this human neoplasia that remains, anyhow, despite the possibility of prevention, the second most common neoplasia in women worldwide [5]. In developed countries, where women undergo regular cytologic screening, the cost of screening, follow-up, and treatment is very high (in the United States of America, it is approximately $5 billion dollars per year).

In conclusion, according to recent world estimates, around 300 million women are carriers of HPV-DNA without clinical expression while approximately 6% of the estimated 9 million cases per year of cancer worldwide may be attributed to HPV infection [4].

This is the time for medical societies and public health entities to consider this phenomenon again, to define and develop strategies for its implication in a preventative and clinical role. Different international organizations such as the World Health Organization and the International Agency for Research on Cancer, in fact, recognize the need to develop a vaccine against human papillomavirus.

What is the anti-HPV vaccine?

The prevention of exposure to, and infection by, high-risk oncogenous genotypes through vaccination could become the most effective and feasible intervention in this pathology. As occurred for the disease caused by the Hepatitis B virus, intense efforts are presently being made in the development of vaccines which could prevent the infection and reinfection from HPV and, presumably, also prevent the development of cancer. To radically interrupt the transmission of this virus, contemporary vaccination of both the female and male population during adolescence (i.e., before the start of sexual activity) would be indispensable.

The presence of viral oncogenes in the human papillomavirus genome poses a theoretical obstacle in the development of a prophylactic anti-HPV vaccine carrying viral DNA. For this reason, efforts have been aimed at the development of a sub-unit vaccine.

The L1 major structural protein, expressed in eukaryotic and prokariotic cells, self-assembles into virus-like particles (VLPs), thus allowing the production of high preparative amounts of VLPs. Since L1 VLPs closely resemble the conformation of authentic virions, their administration induces high levels of papillomavirus-neutralizing IgG antibodies which tend to be type-specific.

Immunoprophylaxis with DNA-free VLPs (empty capsids) has proven to be effective in animal models where experimental studies have provided encouraging results in terms of a satisfactory species-specific immunization of a relatively long duration and passively transmittable. Unfortunately, efficacy trials regarding protection from sexually transmitted diseases in man cannot be tested in animal models [6].

There are different approaches to the anti-HPV vaccine: primary vaccines or primary prevention vaccines which prevent the initial infection; secondary vaccines or secondary prevention vaccines which prevent progression of cervical lesions; and therapeutic vaccines which allow elimination of the residual disease after treatment of SIL or invasive cancer, delay progression of the disease, and induce regression of the disease.

Prophylactic vaccines are composed of virus-like particles, obtained from L1 or L1/L2 structural antigens through recombinant DNA technology. The first generation vaccines contain L1-VLPs or L1/L2 VLPs. Different preparations composed in this way have been tested in man so far to evaluate their efficacy and tolerability. In one study sponsored by the National Cancer Institute (NCI), a vaccine composed of HPV 16 VLP

administered to 72 healthy volunteers (58 women and 14 men) during phase I, induced high levels of neutralizing antibodies after intramuscular injection of three 50 mg doses (day 1, months 1 and 4). The mean antibody response was 40 times greater than that observed after natural infection. The vaccine was well tolerated and the only significant adverse effect was transient pain at the injection site (7). Another phase II study conducted on 100 young healthy women vaccinated with 50 μg doses without adjuvant showed an elevated and consistent immunogenicity [8].

Recent literature reported data on the use of a vaccine containing HPV 11 VLPs which, after the administration to 65 volunteers (24 men and 41 women, with a mean age of 30 years) of three doses of preparation (9, 30, and 100 mg/dose) provoked the production of elevated IgG titres. The vaccine was well tolerated and immunogenic in all cases, with the most frequent side-effect being pain at the injection site [9]. The therapeutic effect of this same type of vaccine in cases of genital condyloma will require further follow-up [10].

The potency of a vaccine composed of HPV-11 VLP administered intramuscularly using four different doses (10, 20, 50 and 100 μg) over a period of six months (at months 0, 2 and 6) to 30 HPV seronegative, healthy female volunteers with negative history of any HPV-correlated disease was recently evaluated. In all subjects who received the vaccine, an HPV-11 VLP specific type Th1 and Th2 positive immunoproliferative response was seen at the third month, following the second injection of the vaccine, and a HPV-11 VLP specific antibody response following the first immunization. This reaction was not observed in the subjects who received placebo [11].

In another very recent double-blind, multicenter study conducted on 2,392 young healthy women (between 16 and 23 years of age), the efficacy of a HPV-16 L1 VLP vaccine was assessed in preventing persistent HPV-16 infection, and then, in a second analysis, in preventing the onset of cervical cancer and its precursors. The most common reason for exclusion from the study was evidence of HPV-16 infection upon enrollment in the study. The study included 631 women who received placebo and 619 women who were given three 40 mg doses of vaccine at day 0, month 2, and month 6. The incidence of persistent HPV-16 infection was 3.8 per 100 women/years at risk in the placebo group and 0 per 100 women/years at risk in the vaccine group (100% efficacy, 95% confidence interval: 90-100; $p < 0.001$). All 41 cases of persistent HPV-16 infection occurred in the placebo group: 31 cases of persistent infection without correlated CIN, five cases of infection correlated to CIN 1, four cases of infection related to CIN 2, and one HPV infection occurring before the patient was lost to follow-up. Of the women who received the vaccine, 99.7% seroconverted specifically for HPV-16. In this group of patients, the prophylactic efficacy of three doses of this vaccine in reducing the incidence of HPV-16 infection persistence and the possibility of reducing the incidence of cervical cancer associated with this serotype of HPV are evident [12].

Despite encouraging results from preclinical and clinical studies, it is very difficult to predict the efficacy of prophylactic vaccines. Considering that the oncogenous virus is sexually transmitted (no animal model allows the study of this event), the relationship between the immune response (neutralizing antibodies and cytotoxic T-lymphocytes) in serum or in cervical mucous and protection in regard to "natural" sexual transmission of the virus at the genital level is unable to be determined.

Therapeutic vaccines may consist of peptides, proteins, chimeric VLPs, DNA, viral vectors, bacterial vectors, dendritic cells, or modified tumor cells. Second generation vaccines are chimeric proteins (VLP, i.e. the association of one of the L1 or L2 structural proteins fused with an E6, E7, or E2 non-structural protein) capable of inducing both a humoral (IgG neutralizing) and a cell-mediated type (cytotoxic T-lymphocytes, CTL) immune reaction.

Capsid proteins are not detectably expressed in the basal cells of squamous epithelium where the viral infection is maintained. For this reason, the immune reaction induced by L1 VLPs is supposedly not able to cause regression of a neoplastic lesion of the uterine cervix. To increase the therapeutic potential of VLP vaccines, polypeptides encoded by non-structural genes of the virus, generally expressed in the basal layer of the cervical epithelium, have been incorporated in the VLP through genetic fusion with both L1 and L2 proteins. This type of combination could increase the efficacy of the vaccine and the duration of immune protection. The ideal role of chimeric vaccines would be that of secondary prevention through elimination of the initial lesions induced by viruses which have escaped neutralization. This effect would also have therapeutic sig-

nificance in regard to residual disease (after treatment of intraepithelial or invasive lesions). Chimeric VLP vaccines containing HPV-16 E7 polypeptide have induced a potent cytotoxic T lymphocyte (CTL) response and specific protection in animal models [13, 14].

Therapeutic vaccines composed of HPV E6 and/or E7 of the recombinant live vaccinia virus, or composed of L2/E7 fusion protein, or of E7 peptide are under study in patients with high-grade SIL or advanced cervical carcinoma [15, 16].

Promising results *in vitro* and in animal models with immunostimulating peptides have been closely followed in various phase I/II clinical trials in humans using peptides derived from E7 protein which binds HLA-A*0201 of the major histocompatibility class I complex (MHC I). The phase I/II study by van Driel *et al.* [17] on 19 female patients with uterine cervical cancer in terminal phase and positive HLA-A*0201 reported a stable disease or tumor regression following vaccination in four patients and disease progression in the others. The authors do not report significant side-effects after administration of the vaccine (administration of scalar doses of 100, 300 and 1000 μg) but associate the poor efficacy to the immunosuppressed condition of the female patients [17].

In a phase II study by Khleif and colleagues on 16 patients with advanced cervical carcinoma, peptides E6 and E7 which bind HLA-A*0201 were used, inducing a cytotoxic cell response valid in 11 patients [18].

Muderspach and colleagues, instead, report on their phase I trial of a peptide E7 vaccine on 18 women patients with CIN III and VIN III who were HPV-16 positive and HLA-A*0201 positive. Ten patients showed a significant increase in cytotoxic activity against E7 after vaccination, 12 of the 18 patients showed negativity in the findings for HPV in the pap smear conducted after the fourth vaccine dose, while all the patients showed positivity in detection of HPV by polymerase chain reaction (PCR) conducted in a loop electrocautery excision procedure (LEEP) four months after the vaccine. Clinically, nine patients had partial or complete regression of their lesions even if there was no correlation found between the degree of regression of the lesion and the development of immune responses, although the latter was elevated [19].

One limitation to the use of peptide vaccines for therapeutic purposes is definitely the association with the specificity of the MHC, seeing that those used in the studies are specific for the HLA-A*0201 allele, thus use is needed only in selected patients. Instead, more promising are the *in vitro* and *in vivo* studies in animal models on the use of chimeric vaccines (VPL-HPV), DNA vaccines, and recombinant vaccines (TA-HPV) which present scarse side-effects and a cell-mediated and a much broader cytotoxic response than with the use of only specific peptides [20].

The question still remains, however, as to whether the mechanisms of immune evasion by the tumor, such as those of downregulation and the loss of MHC class I, can lead to the failure of the therapeutic vaccine. It would seem more likely that in the future there will be the use of therapeutic vaccines as immunotherapy adjuvant to surgery and chemotherapy to prevent recurrences rather than as primary therapy of SIL/CIN and of uterine cervical carcinomas [8, 21].

The valence of the vaccine (number of genotypes), route of administration (humoral versus cellular immunity), form (recombinant, DNA vaccine, transgenic edible plant vaccines that express L1 or L2 proteins, attenuated *Salmonella typhimurium* that expresses HPV L1, synthetic vaccinal antigen) and the target population (children, young adults) are all variables of the vaccine presently being studied [8, 21].

What could be the reason for vaccine failure?

In the first place, the virus infects the human organism through the genital mucosa by spreading itself in the body. All preparations are presently administered through the systemic route in man, and it is not known if such an immunization will lead to the production of humoral immunity at the mucosal level, a reaction necessary to contrast the genital infection caused by HPV (IgA). In animal models, the intranasal administration of purified VLPs [22] and the oral administration of food infected with attenuated *Salmonella typhimurium,* which expresses HPV L1, induced the production of type IgA and IgG neutralizing antibodies at the vaginal level [23]. Moreover, considering the genetic variability of tumors, the cellular immunity induced by the vaccine could be unprotective in regard to genetically unstable cervical neoplasia [24].

We do not know if genital mucosa represents an ideal site for the induction of immunoprophylaxis but it is considered that the intravaginal installation of the vaccine ("vaccination of the female genital tract") would be hardly acceptable by adolescent girls, considered a target population of the vaccine [25].

Why get vaccinated?

The motivation for using an anti-HPV vaccine would vary, depending on the country in which it were to be used. In developing countries, where screening for cervical carcinoma is not accessible, the primary objective would be the prevention of invasive cervical carcinoma. On the contrary, in developed countries, the endpoint of vaccine strategy would also be prevention of neoplastic precursors and benign HPV-related disease (condyloma).

To obtain a significant impact on the "HPV disease burden", the vaccine must be polyvalent, i.e., it needs to protect against multiple different types of HPV including the high and low risk oncogenous types. Specifically, since multiple genotypes of HPV can be found in cervical tumors, the type-specific nature of immune protection requires polyvalence of the anti-HPV vaccine in order to prevent the development of the majority of cervical carcinomas (for example, types 16, 18, 31 and 45). It is theorized that in the near future a polyvalent anti-HPV product may become part of a polyvalent vaccine complex against all sexually transmitted diseases (an "anti-STD vaccine"). Critical to the success of a vaccine protocol is the ability to generate an immune response capable of eliminating the disease and persisting over time.

Vaccination represents, in fact, a medical intervention for the prevention of infectious diseases with the highest cost-benefit ratio since the condition of resistance does not signify costs in terms of health expense, loss of working days, psycho-physical costs and permanent damage to the person who gets sick. Still, medical intervention in the absence of the disease does not have the same significance as therapeutic intervention in the presence of disease. Thus, it is necessary to fight the tendency to refuse, or to consider inadequately, a medical intervention such as vaccination whose benefit is seen, not as immediate, but in the perspective of preventing a future disease: cancer.

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