Development of Prophylactic HPV Vaccines

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ABSTRACT

Since several years it has been accepted that persistent infection with certain (so called-high risk: HR) types of Human papillomaviruses (HPV) represents a strong risk factor for cervical cancer. The most frequent HR HPV types 16 and 18 account for about 70% of this tumour, which is the second most frequent malignancy in women worldwide. Several studies in animal papillomavirus models revealed that protection against infection is conferred by neutralizing antibodies directed against conformational epitopes of the major structural protein L1. Such antibodies can most efficiently be induced by immunization with virus-like particles (VLP) that assemble spontaneously following expression of L1 in recombinant vectors. Large-scale production of HPV 16 and 18 VLPs proved to be successful facilitating, a few years ago, first clinical trials on safety and immunogenicity. In the meantime more than 25,000 women have been included into several efficacy trials which demonstrated protection against persistent infection with HPV 16 and 18 and against the development of precursor lesions to cervical cancer. Although the ultimate proof of success, i.e. reduction of cancer incidence still requires the immunization of large populations and many years of follow-up, the existing data are so persuasive that the responsible agencies in several countries permitted the licensing of the first HPV vaccine in 2006. Several questions such as the duration of protection, the need development of for post-exposure vaccination strategies and availability of such vaccine in low-budget countries are open and will be discussed.

Key words: human papillomavirus (HPV) vaccines, cervical cancer

Preventive vaccines are based on the induction of virus-neutralizing antibodies. For the papillomaviruses this has first been achieved by immunization with formalin-fixed wart extracts and subsequently by inactivated purified viruses¹. In demand of an appropriate infection model, it was difficult to determine the neutralizing activity of serum antibodies. The first in vitro assay for virus neutralization was the inhibition of focus formation of mouse cells in culture by BPV virions². A more sophisticated neutralization assay was introduced by Kreider and coworkers who grafted normal human tissue infected in vitro with HPV 11 or HPV 16 under the renal capsule of immuno-deficient mice where typical lesions develop³. Infection is then monitored by phenotypic analysis and by HPV-specific RT-PCR. Only very recently, a potentially high throughput neutralization assay on the basis of pseudovirions containing a reported gene became available which allows detection of neutralizing antibodies in an in vitro setting⁴. Neutralizing antibodies are directed against the major structural protein L1 and, to a much lesser degree, against the minor capsid protein L2. For induction of neutralizing antibodies, the L1 protein is required to be presented in a correctly folded conformation. This became only possible with the production of L1 virus-like particles (VLPs). In 1986 it had already been shown that purified VP1 of mouse polyomavirus spontaneously assembles into virus-like particles⁵. This inspired in the early 1990s the production of papillomavirus-like particles by different researchers. Zhou and colleagues used a vaccinia virus expression system to produce HPV 16 L1 VLPs, however the yield of this system was extremely low and did not allow further characterization of the antigen e.g. by immunological studies⁶. Shortly after, it was demonstrated that HPV 1, BPV 1 and HPV 11 VLPs can be produced much more efficiently compared to HPV 16 VLPs leading to the speculation whether this might be due to intrinsic properties of the L1 protein of different PV types⁷,⁸,⁹. After a report by Kirnbauer and colleagues it became clear, however, that the HPV 16 L1 gene of the original isolate by Dürst et al. harboured a point mutation rendering its encoded L1 protein virtually assembly defective¹⁰. The HPV 16 prototype genome, used by numerous researchers worldwide

Received for publication January 31, 2007
was derived from a tumour biopsy, in which the HPV 16 genome was integrated into the cellular genome and thus had been without any selection pressure for the maintenance of intact structural genes. When HPV 16 genomes obtained from virus-producing lesions were analyzed, the respective L1 proteins assembled with much greater efficiency into VLPs. Sequence analysis revealed that this difference was based on a single histidine to aspartic acid exchange at position 202 of the HPV 16 L1 protein. VLPs were subsequently produced successfully in insect cells, later also in yeast, in plants and even in cell free systems. Meanwhile, VLPs of several animal and human papillomaviruses have been produced, i.e. cottontail rabbit papillomavirus, rabbit oral papillomavirus, BPV 1 and 4, canine oral papillomavirus and HPV types 1, 2, 6, 8, 11, 13, 16, 18, 31, 33, 39, 45, 56, and 59. For review see16. The production of VLPs in larger quantities made structural and immunological studies possible.

The obtained data from several animal models fostered the concept of prophylactic vaccines against HPV infections. Virus-like particles proved to be prime candidates for prophylactic vaccination since they induce high-titer conformational antibodies of neutralizing capacity both in non-human primates and in man. Pass transfer of IgG from immunized animals it was shown that they confer protection against experimental challenge29.

The first clinical trial with VLPs (of HPV 11) involving human subjects was initiated in 1996 (for reference see Inglis et al.27). HPV 11 VLPs used in this study were produced in insect cells. The success of this trial initiated further HPV vaccine development. In several phase I clinical trials safety and immunogenicity of HPV 11, 16 and 18-specific VLPs generated by expression of the L1 protein in yeast or in insect cells were demonstrated17,19,20–22. Three VLP-based vaccines (HPV 16, HPV 16 + 18, HPV 6 + 11 + 16 + 18) were brought into clinical efficacy trials. The so far only licensed vaccine provided by Merck & Co (Gardasil)30 contains in addition to the most relevant cancer-associated HPV types 16 and 18 also VLPs of the low-risk HPV types 6 and 11 aiming for protection against genital warts whereas the product developed by GlaxoSmithKline has only the two high-risk HPV types. The data obtained from immunization of about 25,000 young women receiving either vaccine or placebo demonstrated after more than 4 years of follow-up protection against incident and persistent infection with HPV 6/11, and/or HPV 16 an 18 and the lesions associated therewith (external genital lesions, high grade CIN, adenocarcinoma in situ)29–32. Longer follow-up and large scale population-based efficacy trials will be required to evaluate the potential of these vaccines to reduce the incidence of cervical cancer33,34. Based on the existing data, however, the regulatory agencies in different countries have approved. It will be more difficult to evaluate the efficacy towards genital warts since the information about the incidence of this disease is rather scarce (http://wrongdiagnosis.com/g/genital warts). The second product manufactured by GlaxoSmithKline (consisting of HPV 16 + HPV 18 VLPs) is expected to enter the market soon.

After introduction of the vaccines there are several open questions that can be solved only after wide application in different countries and longer time of follow-up. The critical issues include the acceptance of the HPV-specific vaccines within different cultures35,36 including the question whether the vaccine will be marketed as preventive measures against a sexually transmitted disease or as anti-cancer prophylaxis37. Other issues are the duration of immune protection and the participation of vaccinated women in Pap-screening programs since HPV 16 and 18 accounts for about 70% of cervical cancer cases38 hence the risk for this disease by other high-risk HPV types is not negligible. Therefore, vaccinated women need to be educated about the necessity to keep up with the screening program although the intervals of the visits can possibly be extended39. Obviously prophylactic vaccination will be highly desirable in areas where screening programs are not offered to the general population and where cervical cancer is a major public health problem. The high price clearly is prohibitive and joint efforts of scientists (developing more cost effective protocols), providers and politicians (discussing multi-tiered pricing) and public and philanthropic foundations (providing large amounts of money) are needed to make the vaccines available to the populations of highest need.

REFERENCES


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RAZVOJ PROFILAKTIČKOG CJEPIVA PROTIV HPV-a

S A Ž E T A K

Od prije nekoliko godina je prihvaćena tvrdnja da trajna infekcija s određenim (tzv. visokorizičnim; HR od engl. high risk) tipovima humanog papilomavirusa (HPV) predstavlja jaki faktor rizika za razvoj raka vrata maternice. Najčešći HR HPV-i su tipovi 16 i 18, povezani s oko 70% ovog tumora, koji je na drugom mjestu najčešćih oboljenja kod žena u svijetu. Nekoliko studija na modelima animalnih papilomavirusa je pokazalo da je zaštita protiv ove infekcije neutralizirajućih antijela protiv konformacijskih epitopa glavnog strukturnog proteina L1. Ta antijela mogu biti vrlo učinkovito inducirana imunizacijom sa česticama sličnim virusima (VLP, engl. Virus-Like Particles) koje se spontano nakupljaju nakon ekspresije L1 u rekombinantnim vektorima. Velika skala proizvodnje VLP-ova od strane HPV 16 i 18 se pokazala uspješnom, olakšavajući tako, prije nekoliko godina, prve kliničke pokuse o sigurnosti i imunogenosti. U međuvremenu, više od 25.000 žena je bilo uključeno u nekoliko kliničkih pokusa o zaštiti protiv trajne infekcije tipovima HPV-a 16 i 18 te o sprječavanju razvoja oštećenja koja prethode raku vrata maternice. Iako je krajnji dokaz uspješna smanjenje stope pojavljanja raka, potrebno je još cijepiti velike skupine te ih godinama pratiti. Ipak, sadašnji podaci su toliko uvjerljivi da su zadužene agencije u nekoliko zemalja dopustile uvođenje prvog cjepiva protiv HPV-a 2006. g. Neka pitanja, kao što je trajanje zaštite, potreba za razvojem strategija nakon cijepljenja i dostupnost ovog cjepiva u siromašnjim zemljama, su još otvorena za diskusiju.