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A long way: History of the prophylactic papillomavirus vaccine

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1. History of papillomaviruses as infectious agents in animals and humans

For more than one and a half centuries, papillomaviruses have been recognized as causative agents for benign and malignant disease in humans as well as in a number of animal species [43]. It took more than five decades to provide sufficient evidence for the general acceptance of HPV as necessary cause for the development of anogenital cancer in humans. At the end of this long road of extensive studies on papillomaviruses by countless scientists worldwide there is finally a prophylactic papillomavirus vaccine at hand. Clearly, the scientific progress in vaccine development was hampered by the fact that papillomaviruses cannot be readily cultivated *in vitro*. In this review we will discuss the path that led to the introduction of the prophylactic vaccine to the public.

Earliest reports about a putative infectious agent being involved in the development of cervical cancers came from observations made by the Italian physician Domenico Rigoni-Stern [51]. He noted that cancer of the womb is found most frequently among women in their fourth and fifth decade and he described behavioural risk factors for this cancer that are still being in the focus of modern epidemiology: age at first sexual intercourse and promiscuity. Despite an imprecise baseline of his study (Rigoni-Stern did not differentiate between cancer of the endometrial and cervical cancer) the link to cervical cancer was later confirmed by other investigators in the 1950s eventually linking cervical cancer to sexually transmitted agents [22,62,63]. In the following, a number of known pathogens were suspected to play a role in cervical cancer development. More refined investigations were subsequently able to rule out an involvement of *Treponema pallidum* (Syphilis) or Herpes Simplex Virus 2 and others in anogenital cancer [66].

Another line of evidence for papillomaviruses being responsible for tumor development was established by experimental transmission of warts and condylomas in man. As early as in the 1890s several investigators induced skin warts in volunteers by the inoculation of hand wart extracts [41,64]. In 1907 Ciuffo demonstrated that a filterable agent is able to induce hand warts [10]. Similar experiments were later performed for condylomas by Waelsch [67]. With the introduction of electron microscopy, virus particles were identified in extracts of hand warts and condylomas. Already in the early 1930s Shope described a virus inducing papillomas in cottontail and domestic rabbits [60]. Shope observed that experimentally induced papillomas in domestic rabbits progress to cancer in high frequency providing the first example of a DNA virus as causative agent for cancers in mammals. Later, Ito and Evans demonstrated that papillomas and tumors can be induced solely with naked viral DNA [33].

In 1976 zur Hausen noted that the epidemiological patterns of cervical cancer and condylomas showed striking similarities [72]. As papillomaviruses had been already tracked down for causing condylomas it was postulated that they might also be causatively involved in the development of cervical cancer. With the onset of molecular biology, a number of genital papillo-

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mavirus types were isolated and cloned into plasmid vectors in the following. HPV 6 DNA was cloned from condylomas, the closely related HPV 11 was isolated from laryngeal papillomas but also detected in genital warts [23,24,59]. With these low-risk papillomavirus genomes at hand, it was possible to prove, for the first time, presence of papillomavirus-related DNA in biopsies of cervical cancer. Shortly after, the two most prevalent high-risk HPV types 16 and 18 were cloned from tumor biopsies [4,15].

2. History of PV vaccination

2.1. Therapeutic approaches

Over the centuries, there have been numerous and sometimes venturesome attempts to treat skin and genital warts which makes it difficult to pinpoint the first therapeutic vaccination against PV. Early observations were made in the course of experimental induction of warts in rabbits and humans. It was noted, that warts develop only in a proportion of the individuals which was interpreted either as inefficiency of the virus to establish infections or 'resistance' of a given individual [26]. Further, it was clear that warts in animals and humans spontaneously regress. During a series of self-inoculations with wart extracts Findlay stated that he became 'immune' to wart induction [21]. It was also observed that local inflammation precedes regression of warts and that warts frequently disappear simultaneously [45]. In the 1930s and 1940s a number of therapeutic vaccination trials were performed based on the injection of autologous and heterologous wart extracts. During his trials, Biberstein inoculated hundreds of patients with filtered extracts from warts and condylomas and was able to record wart regression in the majority of the cases [3]. The likely involvement of the immune system in controlling papillomavirus infections was also underlined by the observation that genital warts but also genital cancer is more frequently found in immuno-suppressed patients [49].

The rabbit papillomavirus model proved to be particular valuable for the study of immune therapy of PV infections. Early studies showed that regression of papillomas and transplanted CRPV-induced tumors can be induced by injection of wart extracts linking papillomas and malignant tumors to the same antigens [18]. In later years, viral antigens responsible for wart regression were identified. The early proteins E1, E2, E6, and E7 protect against virus challenge and induce regression of already existing warts and malignant tumors [28,29]. In recent years a number of efforts have been undertaken to develop therapeutic papillomavirus vaccines some of which have entered clinical trials. It would go beyond the scope of this review to summarize all therapeutic papillomvirus vaccines, most of the approaches are directed to induce cellular immune responses against the E7 tumor antigen (for review see [14,16,61]). Synthetic E7-derived peptides have been analyzed for the treatment of low grade CIN lesion. Anti-E7 immune responses have also been induced by the use of recombinant vaccinia viruses. Interestingly, it was shown that Virus-like particles, the bases for current prophylactic vaccines, are also able to mount strong cellular immune responses likely, because they can directly interact with dendritic, antigen-presenting cells [55,57].

2.2. Prophylactic vaccination

Preventive vaccines are based on the induction of virus-neutralizing antibodies. In case of papillomaviruses this has first been achieved by immunization with formalin-fixed wart extracts and subsequently by inactivated purified viruses [34]. Lacking an appropriate infection model, it was difficult to determine the neutralizing activity of serum antibodies. The first *in vitro* assay for neutralization was the inhibition of focus formation of mouse cells in culture that was, for a long time, limited to BPV virions [53]. Inhibition of hemagluttination, which is based on binding of PV virions to mouse erythrocytes in a receptor-dependent manner, can be achieved by neutralizing antibodies hence this assay is considered a surrogate for measuring inhibition of infectivity [54]. A more complex neutralization assay was introduced by Kreider and coworkers: grafts of normal human tissue infected *in vitro* with HPV 11 or HPV 16 were implanted under the renal capsule of immuno-deficient mice where typical lesions develop [38]. Infection is then monitored by RT-PCR. Only very recently, a potentially high throughput neutralization assay became available which allows detection of neutralizing antibodies in an *in vitro* setting [48].

Neutralizing antibodies are directed against the major structural protein L1 and, to a much lesser degree, also against the minor capsid protein L2. In fact, in an early study *E. coli*-derived L1 protein was used for prophylactic vaccination [50] but was, despite initial promising results not followed up. The reason for the moderate success is that, 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 in form of virus-like particles. In 1986 it had already been shown that purified VP1 of mouse polyomavirus spontaneously assembles into virus-like particles [56]. 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 studies [70].

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 types [27,54,69]. 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 defective [36]. The HPV 16 prototype genome, used by numerous researchers worldwide was derived from a tumor 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 early, 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. 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. cottobtail 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, 58 and 59 [5,9,11–13], for review see [58]. The production of VLPs in larger quantities made structural and immunological studies possible.

The compelling data obtained 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 both in non-human primates and in man [2,8,17,19,20,31, 35,42,47]. Such antibodies proved to have neutralizing capacity [53]. By passive transfer of IgG from immunized animals it was shown that they confer protection against experimental challenge [7].

The first clinical trial with VLPs involving human subjects was initiated in 1996 (for reference see [32]). HPV 11 VLPs were produced in insect cells and at that time laboriously purified by ultracentrifugation, a procedure that is inadequate for large scale production of a human vaccine. Nevertheless, the success of this trial spiked 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 infected with recombinant baculovirus were demonstrated [2,17,19,20,31]. Three VLP-based vaccines (HPV 16, HPV 16 + 18, HPV 6 + 11 + 16 + 18) were already brought into clinical efficacy trials. It should be noted that the vaccine provided by Merck & Co (Gardasil^{TM}) 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 targets only the two high-risk HPVs. The data obtained from immunization of about 25,000 young women receiving either vaccine or placebo demonstrated after 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) [1,30,37,44, 65]. It is as yet unclear to what extent the protection is HPV type-specific. Obviously it would be beneficial for the overall efficacy if the reactivity is not only restricted to HPV types included in the vaccine. 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 cancer [6,40]. Based on the existing data, however, the regulatory agencies in different countries have approved the first vaccine, the second product is expected to be licensed soon. 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 question of acceptance of HPV-specific vaccines within different cultures also needs to be addressed [39, 71]. This includes the issue whether the vaccine will be marketed as preventive measures against a sexually transmitted disease or as anti-cancer prophylaxis [46]. Other issues are the duration of immune protection and the participation of vaccinated women in Pap-screening programs HPV 16 and 18 account for about 70% of cervical cancer cases [68] hence the risk for this disease by other high-risk HPV types is far from 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 extended [25]. 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.

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