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Review Article

HPV vaccines: their pathology-based discovery, benefits, and adverse effects☆

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ABSTRACT

The discovery of the human papillomavirus (HPV) vaccine illustrates the power of in situ–based pathologic analysis in better understanding and curing diseases. The 2 available HPV vaccines have markedly reduced the incidence of cervical intraepithelial neoplasias, genital warts, and cervical cancer throughout the world. Concerns about HPV vaccine safety have led some physicians, health care officials, and parents to refuse providing the recommended vaccination to the target population. The aims of the study were to discuss the discovery of HPV vaccine and review scientific data related to measurable outcomes from the use of HPV vaccines. The strong type-specific immunity against HPV in humans has been known for more than 25 years. Multiple studies confirm the positive risk benefit of HPV vaccination with minimal documented adverse effects. The most common adverse effect, injection site pain, occurred in about 10% of girls and was less than the rate reported for other vaccines. Use of HPV vaccine should be expanded into more diverse populations, mainly in low-resource settings.

1. Introduction

Vaccination is the most successful method to control infectious diseases in terms of both cost and effectiveness. Several studies have demonstrated that both the quadrivalent and bivalent human papillomavirus (HPV) vaccines are safe [1–3], with long-term durability (at least 6 years) for protection against primary infections for the targeted types of HPV viruses and a moderate degree of cross-protection against some nontargeted types. However, to date, there are several controversies surrounding compliance, at times involving government health agencies.

It is important to emphasize that HPV vaccines are not a treatment for HPV-associated diseases that exist at the time of vaccination, nor will it invariably protect against diseases that are caused by types of HPV not covered by the vaccines. Despite the efforts by public health agencies in the United States, the percentage of young women and men who receive the HPV vaccination remains low. Only 33.4% of female adolescents aged 13 to 16 and 6.8% of males had received the 3 recommended HPV vaccine doses in 2012 [4]. In comparison, in Australia, by 2009, 83% of young women had received at least 1 dose and 70% had received all 3 doses of the Gardasil vaccine, with a concomitant decrease in the detection of cervical disease associated with the 4 HPV types from 28.7% before vaccine availability to 6.7% [5]. In June of 2013, the Japanese Ministry of Health partially suspended its HPV vaccination program [6], which demonstrates that immunization programs can be seriously compromised by safety and possibly political concerns. However, much of the published scientific data regarding the safety of HPV vaccines have indicated that the vaccine is very safe. Indeed, a study by a group tracking girls in Denmark and Sweden and based on almost 300,000 people who received almost 700,000 HPV vaccine doses (Gardisal) documented that there were no autoimmune, neurological, or venous thromboembolic adverse affects associated with the vaccine [7].

This review aims to examine current evidence regarding the efficacy as well as the adverse effects of the HPV vaccines after a discussion on how the HPV vaccine was developed in which the important role of diagnostic surgical pathology in this process will be stressed.

2. Observations that led to the development of the HPV vaccine

Human papillomavirus was shown to be the cause of genital warts and cervical intraepithelial neoplasia (CIN) lesions in the 1980s [8]. At that time, it was widely believed that the virus could not be eradicated from the cervix even with effective treatment. Of course, HPV also caused disease at nongenital sites. Indeed, HPV type 2 is the cause of the common verruca that typically localizes to the hands, fingers, and face. It was known for many years either that verruca could spontaneously regress or that nontreated verruca could regress after cryotherapy

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of a dominant lesion [8]. Although this implied that the body may have developed an effective immune response against HPV 2, it was assumed by many that this would not apply to the HPV in cervical lesions.

In the early 1980s, the first successful attempts to detect viral DNA and RNA in situ were documented [8]. The in situ detection of viral DNA moved ahead rapidly and allowed surgical pathologists to use formalin-fixed, paraffin-embedded tissues to study viral-related diseases. Not surprisingly, because HPV is the most common sexually transmitted disease, much attention was focused on the in situ detection of HPV especially in CIN lesions and cervical cancer. The field was greatly aided by the explosive development of molecular pathology that allowed one to simply clone viruses and prepare large amounts of their DNA. Because HPV is a small virus (about 8000 base pairs), it was particularly easy to isolate new HPV types. The definition of a new HPV type was that it had to overall share less than 50% homology with all known HPV types. It should be stressed that all genital tract HPV genotypes share some homology over parts of their genome. This can be exploited to find one HPV type using another HPV type as a probe by allowing the stringency conditions to detect related but distinct types. Indeed, by 1989, more than 55 HPV genotypes had been discovered and available to researchers, including those interested in doing HPV in situ hybridization [8]. At this time, several commercial laboratories, such as ONCOR and Enzo Biochemistry, offering HPV in situ hybridization kits for the surgical pathologist.

In the 1980s and 1990s, a common treatment of CIN lesions was cryotherapy. This involved using a large, cone-like probe that would cover the entire transformation zone and part of the portion of the cervix. The probe would then be brought to about −20°C and left on the cervix for a few minutes, after which the cervical tissue was completely frozen. Because many CINs, especially CIN 1 lesions, have abundant viral capsid proteins L1 and L2, the end result was that the lesion was destroyed and that these viral proteins were now directly exposed to the host's immune system.

It was well documented that about 10% to 15% of women with CIN treated with cryotherapy developed a subsequent CIN lesion over the next few years [8,9] Again, it was assumed that these recurrences were due to persistent HPV virus not destroyed by the cryotherapy. However, in 1990, an article was published in *JAMA* that specifically addressed the question of whether the HPV type in the primary CIN was the same type that was present in the CIN that developed after cryotherapy [9]. The answer was that, in each case, the HPV type in the second CIN was invariably different from the HPV type present in the initial lesion. At around this same time, women with AIDS were unfortunately becoming more common; and CIN/cervical cancer was especially prevalent in these patients. An equivalent study of recurrent CINs after cryotherapy was done in AIDS women. This study showed that, in each case, the initial CIN lesion and the one that formed after cryotherapy contained the SAME HPV type [10]. These observations suggested that one could indeed mount an effective HPV-type–specific immunity after cryotherapy. This, in turn, led to the conclusion that one could develop a vaccine that would protect men and women from the HPV types included in the vaccine.

Representative examples of the pathology and in situ viral detection in CIN lesions are shown in the Figure. Note that the HPV type in the immunocompetent woman in the first lesion was HPV 31. However, the second CIN1 that appeared about 1 year after the cryotherapy was negative for HPV 31 yet strongly positive for HPV 51. It should be added that the commercially available HPV cocktail for these HPV types includes HPVs 31, 33, and 51. One needs to use the individual probes of HPVs 31 and 51 to show that this woman's recurrent CIN had a different HPV type when compared with the primary lesion. Also note the strength of the HPV in situ hybridization signal for each HPV 31 and HPV 51. The strength of the HPV in situ hybridization signal depends on 3 variables: (1) the amount (copy number) of HPV DNA in the infected cells, (2) the concentration of the HPV probe, and (3) the stringency conditions [8]. Under high stringent conditions and using the correct HPV probe concentrations, one can easily differentiate HPV types such as HPVs 31 and 51.

Still, there is much more one can learn about the virology of CIN from the histologic analysis. Note that the cytologic changes of early HPV infection that include well-defined perinuclear halos and nuclear atypia (so-called koilocytes) are most abundant towards the surface of the lesion. Also note that viral DNA and, by extension, viral RNA and capsid proteins are by far most abundant at the surface of the lesion. This highlights the fact that the classic "koilocytotic atypia" is caused by an early and productive HPV infection where the large amount of viral proteins in the upper epithelia makes it possible to induce a type-specific immunity by subjecting the infected epithelia to severe and sudden freezing. It certainly makes teleological sense that HPV DNA/RNA/protein proliferation is most abundant at the surface of the cervical lesion because this will facilitate the spread of the virus via unprotected sexual intercourse.

The Figure also reminds us that the best way to histologically differentiate acute HPV infections of the cervix from its mimics is to recall that the HPV infection causes a disorganized cell growth pattern, whereas mimics of HPV infection show a more uniform pathologic pattern. Specifically, note that in CINs, the perinuclear halos and the nuclei vary in size and shape and that the area shows increased number and disorganized growth pattern of the cells. In comparison, the adjacent normal epithelia at the transformation zone are very inflamed; so the lesion is atypical. But note the uniform-sized and -shaped halos and uniform nuclear details. The HPV negative in situ hybridization result for this tissue underscores the value of HPV in situ hybridization for these histologically equivocal cases.

These observations led to attempts to develop an HPV vaccine because they showed that a given patient could effectively prevent reinfection by the same HPV type after cryoablation of the primary lesion. However, it is very difficult to grow HPV in culture. A major breakthrough in this regard was the ability to synthesize large amounts of hollow viruslike particles that were made from recombinant HPV capsid proteins typically produced by yeast in culture. This allowed one to generate large amounts of the purified antigen that would be the basis of the vaccine.

The type-specific immunity noted in the *JAMA* publication [9] implied that HPV vaccines would need to be made against most of the HPV types that can infect the cervix. Fortunately, HPVs 6 and 11 are found in more than 95% of genital warts; and HPVs 16 and 18 are detected in the majority of cervical cancers. Thus, one could protect more than 95% of men and women from genital (and anal, plus oral) warts and more than 50% of men and women from penile, anal, and cervical cancer by using the Gardasil HPV vaccine. Still, as we will see, although there are some cross-protections with HPV vaccines, owing to conserved areas of L1 among different HPV types, a recurring theme will be the need to generate specific immunity against as many types as possible given the relatively low cross-protection against types not present in the vaccine. Indeed, this recently led to the FDA approval in December 2014 of Gardasil 9, which directly protects against HPVs 6, 11, 16, 18, 31, 33, 45, 52, and 58 [11].

3. Vaccine efficacy

As previously noted, Australia was the first country to provide all young women the HPV vaccine and to make it easy to be vaccinated. The end result was that more than 80% of young girls had received at least 1 dose and 70% had received all 3 doses by 2007. At this stage, a comparison was made of the prevalence of cervical disease caused by HPVs 6, 11, 16, and 18 in the population just before the use of the HPV vaccine and in 2011. The results were dramatic, with the baseline value of 28.7% before vaccine availability decreasing to 6.7% [5].

One of the first large studies that addressed the efficacy of the Gardasil vaccine was published in the *New England Journal of Medicine* in 2007. More than 5000 women were studied; and the rate of HPV