Raising Expectations For Subunit Vaccine

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Multidose regimens are recommended for all prophylactic subunit vaccines. Recent findings from clinical trials of an human papillomavirus virus-like particle vaccine suggest that it may be possible to develop effective single-dose subunit vaccines. The broad implications of these findings are discussed, and the importance of antigen structure and adjuvant in achieving this goal is considered. In conclusion, we argue for the inclusion of single-dose arms in future trials of vaccines, especially if they are based on induction of antibodies by virus-like displayed antigens.

Keywords. HPV; HBV; prophylactic vaccine; virus-like particle; antibody; plasma cell; memory B cell.

Prophylactic vaccines can be divided into 3 main categories: those that contain live attenuated microbes, those that contain killed microbes, and those that contain ≥1 type of microbial subunit [1]. The latter are composed of 1 or a small number of microbial components that are purified and usually delivered with an immunostimulating adjuvant. There is increasing interest in one class of subunit vaccines, virus-like particle (VLP) vaccines, in large part because of the success of vaccines composed of human papillomavirus (HPV) VLPs, which are nonenveloped icosahedral arrays of 72 L1 pentamers. In the phase 3 efficacy trials of the 2 HPV vaccines that were subsequently approved by the Food and Drug Administration, both the quadrivalent vaccine (Gardasil; Merck) and the bivalent vaccine (Cervarix; GlaxoSmithKline) induced almost complete protection from persistent incident infection and high-grade cervical dysplasia associated with the HPV types targeted by the vaccines [2]. These trials, which involved 3 intramuscular injections of VLP vaccine over 6 months (ie, 2 priming doses followed by a booster), also demonstrated the consistent induction of high titers of serum-neutralizing antibodies that, after an initial decline over the following year or so, stabilized at plateau levels that have now been maintained for >8 years [3]. The latter finding, which has occurred in conjunction with continuing strong protection against incident infection and disease, is important because prophylactic vaccines are generally acknowledged to function primarily by the generation of protective antibody responses [4]. The stability of the plateau levels with the HPV vaccines, which presumably reflects the successful induction and persistence of long-lived antibody-secreting plasma cells, is in contrast to findings for other subunit vaccines, such as tetanus toxoid and diphtheria toxoid vaccines, in which antibody titers continue to decline [5]. The stabilization of the VLP-induced antibody titers at levels that are associated with protection leads to an optimistic projection for the long-term efficacy of the HPV vaccines.

Licensed subunit vaccines are routinely administered using a prime/boost strategy of ≥2 doses or, more often, ≥3 doses. Therefore, recent findings from a post hoc analysis of the young women (18–25 years old) who received 1 dose of the bivalent vaccine in a National Cancer Institute–sponsored double-blinded HPV vaccine trial in Costa Rica were unexpected. In this trial, a single priming dose of the vaccine, which targets HPV16 and HPV18, was able to induce stable serum VLP antibody titers against both HPV types in 100% of the recipients who were seronegative at entry, with a geometric mean titer (GMT) that was only 4-fold lower at the end of the 4-year study than the GMTs induced by 2 or 3 doses [6]. The quality of the antibody response after 1, 2, or 3 doses also appeared to be surprisingly similar, in that individual titers of virion-neutralizing antibodies and of VLP-binding antibodies were highly correlated, and the ratios of the 2 titers were similar for each of the dosing regimens. It is unlikely that natural exposure to HPV16 and HPV18 virions contributed substantially to the durability of the antibody responses after 1 dose, because a transient increase in titer, as would be expected if natural exposure were boosting the response, was rarely observed in the recipients, except for the response during the vaccine induction period [6].

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It is difficult, even in principle, to imagine how the post hoc nature of the analysis might be skewing the serologic observations. The finding of persistent antibody responses after a single dose of the bivalent vaccine supports Amman and Slifka's so-called imprinted lifespan model of plasma cell longevity [7]. This model postulates that the strength of the signals that the B cell receives during its initial encounter with antigen determines the duration of plasma cell survival.

Although the Costa Rica vaccine trial was not randomized by number of doses, it is noteworthy that the vaccine efficacy against persistent infection by the vaccine-targeted HPV types was not significantly different for women receiving 1, 2, or 3 doses [8]. Fifteen of 188 subjects (8%) who received 1 dose of the control vaccine (hepatitis A) became persistently infected with HPV16/18 during the follow-up period, whereas none of the 196 subjects who receive 1 dose of Cervarix became persistently infected. Protection after 1 dose, despite the induction of lower neutralizing antibody titers after 4 years, might not be surprising, as studies using a mouse cervicovaginal challenge model found that levels of passively transferred immune sera that were too low to be detected in an in vitro neutralizing assay were protective against in vivo challenge [9]. The planned long-term follow-up of the trial subjects should help determine whether antibody levels and protection continue to persist.

These results challenge the prevailing dogma that subunit vaccines require a prime/boost regimen and suggest that the HPV VLP vaccines should become the benchmark against which future subunit vaccines are judged. In our opinion, the findings to date are sufficiently encouraging to justify a randomized HPV VLP vaccine efficacy trial that includes a 1-dose arm, to rule out the possibility that unknown confounding factors might influence the efficacy seen after 1 dose. Positive long-term efficacy results in such a trial could, if coupled with stable antibody titers, have far-reaching implications for future development of other subunit, as well as killed virus, vaccines. The development of effective single-dose vaccines would be transformative for vaccine implementation efforts, particularly in low-resource settings, where many vaccines remain underused.

Several factors could explain why stabilization of high-titer antibody responses after 1 dose has not been observed in clinical trials of other subunit vaccines. One possible explanation is that 1-dose recipients were simply not followed up in most trials, because of the expectation that they would not respond well. However, the most critical factor contributing to the unexpected results is likely to be the specific structural characteristics of the antigen in the HPV vaccines. As first proposed by Bachmann and Zinkernagel, B cells appear to have evolved to recognize, via their B-cell receptors (BCRs), the dense repetitive display of epitopes at 50–100-Å spacing as foreign or dangerous, leading to exceptionally strong B-cell activation and survival signals [10]. This density of surface antigen is found on most virions and on other microbial surface structures, such as bacterial pili, but is not generally found on mammalian surfaces that are routinely exposed to the systemic immune system. Virus-like display of self antigens at this spacing can even break B-cell tolerance to self, leading to high-titer auto-antibody responses in animal models and, more recently, in human trials [11, 12].

The HPV vaccine may be the first subunit vaccine with true virus-like display of surface epitopes to be stringently evaluated in humans. Simple toxoid and carbohydrate conjugate vaccines clearly do not have this molecular pattern. The virions on which inactivated virus vaccines, such as hepatitis A and polio, are based have this epitope spacing. However, it is likely that the critical pattern is disrupted by the inactivation process, which involves the cross-linking of surface proteins (eg, by formalin treatment). This hypothesis raises the possibility that the B-cell immunogenicity of killed viral vaccines might be similar to that of the HPV VLPs, if the killed vaccines were subjected to an inactivation process, such as hydrogen peroxide treatment, that did not disrupt their surface structure [13]. Live attenuated virus vaccines, such as yellow fever or vaccinia, can induce essentially lifelong antibodies after a single dose [7]. The results from the HPV vaccine trial raise the question for live virus vaccines of whether display of their surface antigens in a natural high-density pattern may be more critical for this response than the fact that they are infectious.

While display of the antigen in a dense repetitive array appears to be the best method thus far for generating durable antibody responses in humans, several unanswered questions remain for translating the implications of the HPV VLP vaccines to those against other infectious agents. One question is whether an adjuvant containing a Toll-like receptor (TLR) agonist is required. The bivalent vaccine contains monophosphoryl lipid A, a TLR4 agonist, in addition to an aluminum salt [14]. Signaling through a TLR may augment the BCR signals, T helper cell responses, and perhaps other innate immune signaling in promoting plasma cell survival [15]. It will be interesting to determine whether the quadrivalent vaccine behaves similarly after a single dose, as it contains only an aluminum salt adjuvant. A second question is whether VLPs based on enveloped viruses can work as well. The hepatitis B vaccine is composed of the hepatitis B virus surface antigen (HBs) floating in a lipid bilayer [16]. HBs titers wane after the standard 3-dose regimen, and many vaccinees become seronegative over time [17]. The first dose of the hepatitis B vaccine elicits memory B cells in most individuals but limited serum antibodies. In one study in healthy young adults, vaccine-induced HBs antibodies were detected at 12 months in only 21% of subjects who received a single dose of vaccine [18]. It is possible that linkage of the envelope protein to a core or matrix structure, as commonly occurs in authentic enveloped virions, might

generate a more rigid display of the target antigen, leading to stronger B-cell activation.

A third question is whether virus-like display is as superior for inducing memory B cells as it is for inducing long-lived plasma cells. Preexisting antibodies seem to be critical for the high efficacy of the HPV VLP vaccines, as they appear to generate sterilizing immunity in most subjects, and the vaccine-induced responses do not hasten the clearance of prevalent infection [19]. Malaria vaccines that are designed to prevent liver infection by the transient sporozoite form of the parasite would also likely require sufficient titers of preexisting antibodies to protect from disease [20]. In contrast, antibody titers to hepatitis B vaccines often fall below detectable levels, yet the vaccines protect against hepatitis B–associated liver disease, even in individuals who seroconvert for nonvaccine hepatitis B virus antigens, presumably by ensuring an effective anamnestic response by memory B cells [21]. A single dose of the hepatitis B vaccine primed an excellent memory response when subjects were boosted 4 years later, despite the apparently poor induction of long-lived plasmid cells [18]. Thus, the efficiency of inducing 1 arm of antibody memory may not predict induction of the other arm. Therefore, for some disease targets, it would be helpful to determine whether virus-like display vaccines are substantially better at inducing memory B cells than less complex subunit vaccines, particularly after a single dose.

Finally, we believe that the HPV vaccine should have sufficiently raised the expectations of the performance of prophylactic vaccines to warrant a reconsideration of some basic outlines of clinical trials of new vaccine candidates, regardless of the microbial target, especially if protective immunity is thought to depend on long-lived plasma cells. In phase 1 or 2 trials, it would now seem advisable to include a single-dose arm whose subjects are followed for at least 2 years, to obtain a reasonable indication of whether the durability of the antibody responses matches that of the HPV vaccine benchmark. If it does not, it could be worth considering whether to reengineer the immunogen to be more virus like and/or to include an immunostimulator, such as a TLR agonist. If the immunogenicity results are promising, then inclusion of a 1-dose arm in efficacy trials should be considered. At present, regulatory authorities may ask for evidence that a novel adjuvant is critical in the formulation of a new vaccine candidate. In the future, regulatory authorities and/or public health officials may consider asking the, until recently, improbable question of whether a booster dose is necessary.

Notes

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Potential conflict of interest. J. T. S. and D. R. L. are inventors on US government–owned patents related to papillomavirus VLP vaccine technology that are licensed to Merck and GlaxoSmithKline.

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