Induction of immune memory following administration of a prophylactic quadrivalent human papillomavirus (HPV) types 6/11/16/18 L1 virus-like particle (VLP) vaccine

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Abstract

Background: The duration of protection afforded by vaccines represents a critical test of their utility as public health interventions. Some vaccines induce long-term immunity, while others require booster doses. Vaccines that induce long-term protection are usually characterized by the generation of immune memory. Recent trials of a quadrivalent (types 6, 11, 16, 18) human papillomavirus (HPV) vaccine have demonstrated high efficacy through 5 years of follow-up. We evaluated the extent to which the vaccine is able to generate HPV type-specific immune memory.

Methods: A total of 552, 16–23-year-old women were enrolled in a double-blind, placebo-controlled study. At enrollment, subjects were randomized in a 1:1 ratio to receive three-dose regimens of quadrivalent HPV vaccine or placebo with 3 years’ follow-up. A subset of 241 subjects (n = 114 in the quadrivalent HPV vaccine group and n = 127 in the placebo group) underwent 2 further years of follow-up. All extension subjects received quadrivalent HPV vaccine at month 60 to examine the extent of immune memory in response to the primary vaccination series.

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1. Introduction

Human papillomavirus (HPV) infection is associated with cancers of the cervix, vulva, vagina, anus, penis, and the oropharynx [1–5], as well as genital warts [6]. Data suggest that within 3 years after initiation of sexual activity, up to 48% of women will have evidence of cervical HPV infection [7]. Infection with HPV is considered to be a requisite step in the development of cervical cancer [8]. Thus, HPV DNA is found in the cervix of over 99% of all women with cervical cancer [8].

Prophylactic HPV vaccination represents a promising strategy to prevent the occurrence of cervical cancer and other HPV-related diseases. Administration of quadrivalent HPV (types 6, 11, 16, 18) L1 virus-like particle (VLP) vaccine (GARDASIL®, Merck & Co., Inc.) to 16–26-year-olds induces potent anti-HPV 6, 11, 16, and 18 responses. Anti-HPV levels in response to immunization with quadrivalent HPV vaccine have been shown to persist in vaccinated subjects through 4.5 years post-vaccination [9]. The vaccine was highly effective through 5 years post-enrollment, with no breakthroughs due to waning immunity [10]. What has not yet been determined, however, is how long protective antibodies generated in response to immunization with quadrivalent HPV vaccine will last. This is an important question, as women (and men) remain at risk for HPV infection as long as they are sexually active.

The duration of protection afforded by vaccines can vary. A three-dose regimen of Hepatitis B (Hep B) vaccine has been shown to provide immunity for a period of at least 20 years. On the other hand, a three-dose regimen of Diphtheria–Tetanus–Pertussis (DTaP) requires boosting at 5–10-year intervals through late adolescence.

One of the hallmarks of vaccines that confer long-term immune protection is the development of immune memory, which is defined as vaccine-induced generation of long-lived memory immune cells that, upon re-exposure to the relevant antigen, generate a vigorous immune response that prevents or aborts infection. An example of vaccine-induced immune memory can be seen with Hep B vaccine. Like the HPV vaccine, the Hep B vaccine is composed of a viral surface antigen (Hepatitis BsAg) arranged into virus-like particles, formulated with aluminum-containing adjuvant. This vaccine has been shown to induce an immune response that results in detectable antibody levels for at least 10 years [11,12].

Results: Serum anti-HPV levels declined post-vaccination, but reached a plateau at month 24 that remained stable through month 60. Administration of a challenge dose of vaccine induced a classic anamnestic response, with anti-HPV levels 1 week post-challenge reaching levels observed 1 month following the completion of the three-dose primary series. At 1 month post-challenge, anti-HPV responses were higher than those observed 1-month post-dose 3.

Discussion: A three-dose regimen of quadrivalent HPV vaccine induces high efficacy and stable anti-HPV levels for at least 5 years. Vaccination also induces robust immune memory. These findings suggest that the efficacy of this vaccine will be long lasting.

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informed consent and other statutes or regulations regarding the protection of the rights and welfare of human subjects participating in biomedical research.

The original study was a dose-ranging study of three formulations of the quadrivalent HPV vaccine and two placebo arms with different adjuvant doses (225 or 450 μg) corresponding to these vaccines. After selection of the Phase III dose formulation, the study proceeded to evaluate the efficacy, immunogenicity, and tolerability of this vaccine formulation through 3 years [9,16]. At the completion of the year 3 visit, subjects from Brazil and Europe who had participated in the dose-ranging study and had been vaccinated with either the Phase III quadrivalent HPV vaccine or placebo formulations were eligible for participation in the extension, providing they had not discontinued during the initial 3-year study. Women from the U.S. were not eligible to enroll in the extension phase as they were largely from universities and were finishing school, making participation in an extension study for a further 2 years unlikely. Of the 551 women who were randomized and vaccinated with either the Phase III formulation (which was subsequently approved as GARDASIL®, Merck and Co., Inc) or placebo, 241 were enrolled in an extension of the study to obtain an additional 2 years of follow-up data for safety, efficacy and immunogenicity (5 years of follow-up in total). Efficacy through 5 years has been described [10]. In the same study, at the 5-year mark, an antigen challenge was given to evaluate whether quadrivalent HPV vaccine had induced immune memory. Subjects who originally received vaccine were given another dose at month 60, while placebo subjects received the first dose of a three-dose vaccine regimen.

2.2. Study vaccine

The quadrivalent HPV vaccine consisted of a mixture of four recombinant VLPs, each composed of the L1 major capsid protein of HPV types 6, 11, 16, or 18 synthesized in Saccharomyces cerevisiae [17–19]. The Phase III dose formulation (which is now the marketed dose of the vaccine) included 20 μg of HPV 6 L1 VLP, 40 μg of HPV 11 L1 VLP, 40 μg of HPV 16 L1 VLP and 20 μg of HPV 18 L1 VLP, formulated with 225 μg of proprietary amorphous aluminum hydroxypophosphate sulfate adjuvant in a total carrier volume of 0.5 mL. This adjuvant was shown in preclinical models to be more immunogenic in the context of HPV L1 VLP vaccines than aluminum hydroxide or aluminum phosphate salts. The placebo contained the same adjuvant and was visually indistinguishable from vaccine.

2.3. Clinical follow-up

At the start of the original study, a 0.5-mL dose of quadrivalent vaccine or placebo was administered by intramuscular injections at day 1, months 2 and 6. Serum samples were obtained at day 1 and months 2, 3, 6, 7, 12, 18, 24, 30 and 36. In the extension, 241 subjects from Brazil and Europe underwent 2 further years of follow-up. These subjects underwent additional follow-up visits at months 54, 60, 60 + 1 week, and 61 which included serum anti-HPV testing. In all subjects, adverse experiences were recorded for days 1–15 following any vaccination.

2.4. Laboratory analyses

Serum anti-HPV 6, 11, 16 and 18 immunoglobulin levels were measured using a competitive Luminex immunoassay (cLIA) which were reported in arbitrary units (milli-Merck units per milliliter or mMU/mL) relative to the standard curves generated for each individual HPV type [20]. An audit conducted by Merck Research Laboratories concluded that there was a deviation from the Standard Operating Procedure (SOP) for testing a subset of serum samples from the protocol. Approximately 0.1% of day 1 serology results and 0.4% of post-vaccination serology results were determined to have been tested outside of the Standard Operating Procedure. All day 1 serum samples which were tested out of compliance with the SOP were reanalyzed. The remaining non-conformant test results were removed from the database.

2.5. Statistical analysis

To be eligible for inclusion in any of the three analysis populations used for the immunogenicity analyses in the extension phase of the study, subjects must have received three primary series doses of quadrivalent HPV vaccine or placebo, a dose of the vaccine at month 60 (subjects who originally received vaccine were given another dose at month 60, while placebo subjects initiated a three-dose vaccine regimen) and have valid serology data from the month 61 visit. The month 61 serology data used for the main immunogenicity analysis in the extension study must have been collected within an appropriate day range relative to the month 60 vaccination, but no day range criteria for the vaccinations (primary series or challenge dose) were used to determine eligibility for analysis.

The main analysis population for immunogenicity included all subjects enrolled in the extension, regardless of general protocol violations, who met the above criteria and were seronegative to the appropriate HPV type(s) at day 1 and PCR-negative to the appropriate HPV type(s) at day 1 and through month 60 (extension per-protocol immunogenicity population [PPI]). The extension PPI population included subjects whose primary series vaccinations occurred outside of the day ranges relative to day 1 specified for the primary analysis. Two additional subject populations were analyzed which included all subjects enrolled in the extension, regardless of general protocol violations, who met these criteria: (1) naïve (by serology and PCR) to the relevant HPV type at day 1, and (2) seropositive and PCR-negative to the relevant HPV type at day 1.
2.6. Role of the funding source

The studies were designed by the sponsor (Merck and Co, Inc.) in collaboration with clinical site investigators. The sponsor collected the data, monitored the conduct of the study, performed the statistical analysis and coordinated the writing of the manuscript with all authors. Data were unblinded for statistical analyses after the data were screened for accuracy and completeness, protocol violators were identified and the databases were locked for the primary analysis at month 36 [16]. In the extension, the subject and the investigator, study site personnel, laboratory personnel conducting the clinical assays and the pathology panel used in endpoint adjudication remained blinded to vaccination group. The authors were actively involved in data analysis and interpretation, and approved the final manuscript. All authors vouch for the veracity and completeness of the data and the data analyses.

3. Results

A total of 552 women were randomized to receive either the marketed formulation of quadrivalent HPV vaccine or placebo in the original study (Fig. 1). Efficacy and immunogenicity results for the original study have been previously described [9,16]. A subset of subjects enrolled in the base study (n = 241) from Brazil, Norway, Sweden and Finland were enrolled in an extended follow-up study. Baseline characteristics were similar between treatment groups in both the overall cohort from the base study, and among subjects enrolled in the 2-year extension [10]. Twenty subjects (8 previously given placebo and 12 previously given quadrivalent vaccine) discontinued the study during the extension phase. Reasons for discontinuation included pregnancy (n = 4), subject moving (n = 3), withdrawn consent (n = 3), protocol violation (n = 1) and ‘other reasons’ (n = 9). “Other reasons” included “personal reason” (n = 4), “not interested in continuing” (n = 3), “planning pregnancy” (n = 1) and “unwilling to continue” (n = 1). No discontinuations were determined by the investigator to be related to vaccine.

Administration of quadrivalent HPV vaccine resulted in a substantial immune response as measured by anti-HPV 6, 11, 16 and 18 geometric mean titer (GMT) [9]. The highest anti-HPV GMTs were observed at month 7 (1 month post-dose 3) (Fig. 2). Anti-HPV GMTs declined thereafter, but reached a plateau at month 24 that remained stable through 5 years. Vaccine-induced anti-HPV levels between months 36 and 60 remained at or above those observed among women who were seropositive at enrollment, indicating prior exposure to vaccine HPV types (Fig. 2).

Administration of a challenge dose of quadrivalent HPV vaccine to subjects who were previously immunized with quadrivalent HPV vaccine resulted in potent anti-HPV 6, 11, 16 and 18 anamnestic responses (Table 1). Antibody responses against HPV 6, 11, 16 and 18 were pronounced after antigen challenge, and rose rapidly in the month following vaccination. For example, anti-HPV 18 GMTs rose >20-fold between months 60 and 60+1 week, and >25-fold between months 60 and 61. In comparison, anti-HPV 18 lev-
Fig. 2. Persistence of anti-HPV responses following a three-dose regimen of quadrivalent HPV vaccine or placebo. Data are shown for subjects in the extension per-protocol immunogenicity population who received quadrivalent HPV vaccine in the original study, subjects in the extension per-protocol immunogenicity population who received placebo in the original study, and subjects who were seropositive and PCR negative to vaccine HPV types who received placebo in the original study. Anti-HPV 6, 11, 16, and 18 cLIA results are in panels (A–D), respectively. Error bars represent 95% confidence intervals.

...else rose >20-fold between months 2 and 3 (response to dose 2 of the primary series) and <10-fold between months 6 and 7 (response to dose 3 of the primary series).

Analyses were conducted to evaluate the proportion of subjects who responded to the challenge dose. As shown in Table 2, the great majority of subjects who were given the challenge dose of vaccine at month 60 had higher anti-HPV levels 1 week and 1 month after antigen challenge than those observed at month 60. In addition, a substantial number of subjects had anti-HPV levels that were above the levels that they achieved at month 7. In general, anti-HPV level at month 60 correlated with the strength of the anamnestic response observed following administration of the challenge dose.

A proportion of subjects in the group that participated in the extension and received a three-dose regimen of quadrivalent HPV vaccine in the original study were found to be seronegative to one or more vaccine HPV types at month 60. However, there were no breakthrough cases of confirmed HPV 6, 11, 16 or 18 infection or related disease caused by waning immunity over the 4.5-year post-vaccination observation period (10 new cases were observed in the placebo group during the extension period). These findings suggest that in a proportion of subjects, vaccine-induced protection is mediated by anamnestic responses following exposure. To evaluate whether subjects who were seronegative at month 60 had immune memory, we evaluated the response to the challenge dose among subjects who received a three-dose regimen of quadrivalent HPV vaccine in the original study and who were nominally seronegative to at least 1 vaccine HPV type at month 60 (Table 2). Among vaccinated subjects who were anti-HPV 6, 11 and 18 seronegative at month 60, 75% (6/8), 86% (6/7) and 97% (29/30) became seropositive to the relevant vaccine HPV type 1 month post-challenge, respectively. Among these subjects, 50% (3/6), 83% (5/6) and 76% (22/29) achieved anti-HPV 6, 11 and 18 levels, respectively, at month 61 that were at or above the levels that they achieved 1 month post-dose 3. There was one subject who was anti-HPV 16 seronegative at month 60. This subject became seropositive 1 month post-challenge with an anti-HPV 16 level above that achieved 1 month post-dose 3.

Detailed safety evaluations were conducted in subjects who received the challenge dose. Subjects reported somewhat more adverse experiences within 15 days following antigen challenge than within 15 days post-dose 3 (Table 3). This increase was due primarily to an increase in injection-site adverse experiences, most of which were mild to moderate in intensity. The most common injection-site adverse experiences included injection-site pain (66% post-dose 3...
Table 1

Comparison of HPV antibody responses subsequent to doses at month 7 (after dose 3 of original treatment regimen) of the original study and month 60 (antigen challenge) in the extension per-protocol population.

<table>
<thead>
<tr>
<th></th>
<th>GMT (mMU/mL)</th>
<th>95% CI</th>
<th>Fold change from month 7</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HPV 6</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Month 7</td>
<td>549.2</td>
<td>(460.6, 654.7)</td>
<td>–</td>
</tr>
<tr>
<td>Month 60 (pre-challenge)</td>
<td>67.7</td>
<td>(53.5, 85.7)</td>
<td>–</td>
</tr>
<tr>
<td>Month 60 + 1 week</td>
<td>503.3</td>
<td>(344.2, 736.1)</td>
<td>0.91 (7.4)</td>
</tr>
<tr>
<td>Month 61</td>
<td>693.2</td>
<td>(451.9, 1063.3)</td>
<td>1.3 (10.2)</td>
</tr>
<tr>
<td><strong>HPV 11</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Month 7</td>
<td>635.5</td>
<td>(521.3, 774.9)</td>
<td>–</td>
</tr>
<tr>
<td>Month 60 (pre-challenge)</td>
<td>70.1</td>
<td>(52.5, 93.7)</td>
<td>–</td>
</tr>
<tr>
<td>Month 60 + 1 week</td>
<td>1417.5</td>
<td>(1009.0, 1991.4)</td>
<td>2.2 (20.2)</td>
</tr>
<tr>
<td>Month 61</td>
<td>2652.4</td>
<td>(1956.7, 3595.3)</td>
<td>4.2 (37.8)</td>
</tr>
<tr>
<td><strong>HPV 16</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Month 7</td>
<td>3870.0</td>
<td>(3157.0, 4744.0)</td>
<td>–</td>
</tr>
<tr>
<td>Month 60 (pre-challenge)</td>
<td>404.2</td>
<td>(312.9, 522.1)</td>
<td>–</td>
</tr>
<tr>
<td>Month 60 + 1 week</td>
<td>4466.4</td>
<td>(3095.2, 6445.0)</td>
<td>1.1 (11.0)</td>
</tr>
<tr>
<td>Month 61</td>
<td>5714.0</td>
<td>(3829.7, 8525.4)</td>
<td>1.5 (14.1)</td>
</tr>
<tr>
<td><strong>HPV 18</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Month 7</td>
<td>741.2</td>
<td>(576.8, 952.4)</td>
<td>–</td>
</tr>
<tr>
<td>Month 60 (pre-challenge)</td>
<td>44.7</td>
<td>(31.8, 62.8)</td>
<td>–</td>
</tr>
<tr>
<td>Month 60 + 1 week</td>
<td>1033.2</td>
<td>(753.9, 1415.8)</td>
<td>1.4 (23.1)</td>
</tr>
<tr>
<td>Month 61</td>
<td>1230.0</td>
<td>(904.5, 1672.5)</td>
<td>1.7 (27.5)</td>
</tr>
</tbody>
</table>

GMT: Geometric mean titer; CI: confidence interval; PCR: polymerase chain reaction.

The extension per-protocol population includes all extension subjects who received three primary injections of quadrivalent HPV vaccine, an antigen challenge at month 60, were seronegative and PCR-negative at day 1 to the respective vaccine HPV types, PCR-negative through month 60 to the respective vaccine HPV types, and had valid serology data 4 weeks post-challenge.

versus 78% post-dose 4), injection-site swelling (12% post-dose 3 versus 14% post-dose 4) and injection-site erythema (9% post-dose 3 versus 12% post-dose 4). Approximately 9% of subjects who received a primary series of quadrivalent vaccine reported a temperature ≥37.8°C (100°F) from days 1 to 5 following dose 3 of the primary series at month 6. However, only 3% of subjects experienced a temperature ≥37.8°C (100°F) from days 1 to 5 post-dose 4. The proportions of subjects who reported serious adverse experiences post-challenge and post-dose 3 were comparable.

4. Discussion

The results presented in this report demonstrate that administration of a three-dose regimen of quadrivalent (types 6, 11, 16, 18) HPV L1 VLP vaccine to young women results in a potent immune response characterized by detectable serum type-specific anti-HPV antibodies through at least 5 years and generation of robust immune memory in most subjects.

Immune memory mediated by memory B cells plays an important role in effective immunization. Hallmarks of immune memory include an amplified antibody response (an anamnestic response), decreased lag time to response and increased sensitivity to antigen [21]. Not all responses to antigen will result in immune memory, as this phenomenon is dependant on many precise interactions between the innate and adaptive immune systems.

One of the first steps in the process of memory B cell development involves antigen presenting cells (APCs) such as dendritic cells (or B cells themselves) presenting antigen to naïve T-helper (Th) cells [22]. The nature, dose and persistence of a specific antigen play a key role in whether that antigen can engender immune memory. Also, because only soluble protein antigens are able to activate Th cells, these antigens (as opposed to carbohydrate or other types of antigens) are primarily responsible for the generation of immune memory [23].

Once activated through an interaction with an APC bearing antigen, Th cells will interact with B cells; an interface that leads to the activation of B cells and their transformation into plasma cells [22]. A proportion of activated B cells will become memory B cells, which are characterized by consistent, long-term, low-level antibody production [24]. Because APCs are not required to generate effector cells specific for an antigen to which the body has already been exposed, reintroduction of that antigen will result in rapid, large-scale antibody production from memory B cells. Antibodies generated during an anamnestic immune response generally have a higher affinity for antigen than those generated by the B cells that responded during the primary immune response, further accelerating viral clearance. For an in-depth review see Ref. [22].

Given the critical nature of immune memory to the overall utility of vaccines, and the complexity of the induction of immune memory, a demonstration of the capacity of a new vaccine to induce immune memory requires a
Table 2
Anti-HPV responses among subjects who received a three-dose regimen of quadrivalent HPV vaccine in the original study and were in the extension per-protocol immunogenicity population

(A) Anti-HPV 6 levels

<table>
<thead>
<tr>
<th></th>
<th>All subjects in PPI population for HPV 6</th>
<th>Subjects in the PPI population who were anti-HPV 6 seronegative at month 60</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n m (%) anti-HPV 6 seropositive</td>
<td>m (%) with anti-HPV 6 above month 60 level</td>
</tr>
<tr>
<td>Month 60</td>
<td>79 71 (89.9)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Month 60 + 1 week</td>
<td>79 76 (96.2)</td>
<td>68 (87.2)</td>
</tr>
<tr>
<td>Month 61</td>
<td>80 76 (95.0)</td>
<td>66 (83.5)</td>
</tr>
</tbody>
</table>

(B) Anti-HPV 11 levels

<table>
<thead>
<tr>
<th></th>
<th>All subjects in PPI population for HPV 11</th>
<th>Subjects in the PPI population who were anti-HPV 11 seronegative at month 60</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n m (%) anti-HPV 11 seropositive</td>
<td>m (%) with anti-HPV 11 above month 60 level</td>
</tr>
<tr>
<td>Month 60</td>
<td>79 72 (91.1)</td>
<td>1 (1.3)</td>
</tr>
<tr>
<td>Month 60 + 1 week</td>
<td>79 78 (98.7)</td>
<td>75 (96.2)</td>
</tr>
<tr>
<td>Month 61</td>
<td>80 79 (98.8)</td>
<td>77 (97.5)</td>
</tr>
</tbody>
</table>

(C) Anti-HPV 16 levels

<table>
<thead>
<tr>
<th></th>
<th>All subjects in PPI population for HPV 16</th>
<th>Subjects in the PPI population who were anti-HPV 16 seronegative at month 60</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n m (%) anti-HPV 16 seropositive</td>
<td>m (%) with anti-HPV 16 above month 60 level</td>
</tr>
<tr>
<td>Month 60</td>
<td>82 81 (98.8)</td>
<td>3 (3.7)</td>
</tr>
<tr>
<td>Month 60 + 1 week</td>
<td>81 81 (100.0)</td>
<td>70 (86.4)</td>
</tr>
<tr>
<td>Month 61</td>
<td>81 80 (98.8)</td>
<td>72 (88.9)</td>
</tr>
</tbody>
</table>

(D) Anti-HPV 18 levels

<table>
<thead>
<tr>
<th></th>
<th>All subjects in PPI population for HPV 18</th>
<th>Subjects in the PPI population who were anti-HPV 18 seronegative at month 60</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n m (%) anti-HPV 18 seropositive</td>
<td>m (%) with anti-HPV 18 above month 60 level</td>
</tr>
<tr>
<td>Month 60</td>
<td>85 55 (64.7)</td>
<td>2 (2.4)</td>
</tr>
<tr>
<td>Month 60 + 1 week</td>
<td>84 82 (97.6)</td>
<td>80 (96.4)</td>
</tr>
<tr>
<td>Month 61</td>
<td>86 84 (97.6)</td>
<td>83 (97.7)</td>
</tr>
</tbody>
</table>

The table compares all subjects with subjects who had become anti-HPV seronegative by month 60. n: Number of subjects with non-missing data; m: number of subjects with indicated characteristic. PPI: Per-protocol immunogenicity.

† One subject was seronegative to type 16 at month 60. This subject seroconverted by 1 week post-challenge and continued to have anti-HPV levels above the serostatus cutoff at month 61, with month 60 + 1 week and month 61 anti-HPV 16 levels above that observed at month 7.

demonstration of the development of anamnestic responses following an antigen challenge, as is provided in the present report.

In this report, persistence of antibodies specific for vaccine-related HPV types was documented up to 60 months following initiation of the primary vaccination series. Anti-HPV levels decreased over time from month 7, reaching a plateau at month 24 that remained steady through month 60. Such antibody kinetics is not unexpected. Immune responses typically wane with time after antigen stimulation, largely because the clearance of antigen removes the stimulus for further antibody production. Additionally, plasma cells have a finite lifespan, leaving behind only low-level antibody production from memory B cells.

In response to antigen challenge with quadrivalent HPV vaccine 54 months following completion of the primary three-dose series subjects experienced a rapid increase in anti-HPV antibody production. This suggests that the primary series of vaccinations were able to engender the creation of memory B cells, and that it is these memory cells that are activated to produce antibodies in response to further antigenic challenge with quadravalent HPV vaccine. This hypothesis is supported by the more potent antibody responses as measured by GMT that were seen following antigen challenge. Additionally, subjects receiving quadrivalent HPV vaccine who have been previously exposed to HPV 6, 11, 16, or 18 (seropositive but PCR negative) display GMTs greater than those of subjects who were HPV naïve prior to vaccination.
It is important to note, however, that the route of antigen challenge in this report (intramuscular injection) is not the same as it would be outside of a clinical trial (mucosal contact); thus, exposure to an infected individual may and result in different anamnestic anti-HPV antibody responses.

The minimum protective antibody level required to maintain vaccine-induced protective efficacy depends on the specifics of the antigen, the antibody response to that antigen, the assay used to measure the antibody response and whether nascent infection can be aborted by generation of a timely anamnestic response. Preclinical studies demonstrated that the efficacy of L1 VLP vaccines is mediated by humoral responses. Administration of the quadrivalent HPV vaccine generates a robust response against each of the four vaccine components, but the minimum protective anti-HPV level has not been defined, due to the lack of late confirmed breakthrough infections in the subjects who received the vaccine. The lack of such infections, compared with the continuing infections in the placebo group suggests that the anti-HPV levels induced by the vaccine remained protective for at least 5 years.

The mechanism for protection among subjects who become nominally anti-HPV seronegative several years after vaccination remains to be determined. One possibility is that only minimal levels of anti-HPV are required to protect against infection. This would imply that the assay used is not sufficiently sensitive to measure these levels, even though the validation of the assay demonstrated its high sensitivity [26]. Alternatively, because the assay measures only antibodies that compete with those directed at a single known neutralizing epitope, it is possible that other neutralizing antibodies not detected by the assay can protect against infection. It is also possible that low levels of anti-HPV antibody could inactivate invading HPV virions before they infiltrate the cell, as this process has been shown to be relatively slow [27]. Nascent infection may activate memory B cells which induce an anamnestic response that aborts the infection. However, as it is not known whether the rapidity of the memory B cell response is sufficient to prevent an initial infection, it is possible that both sustained low antibody levels and memory B cells play a role in the protective efficacy engendered by the quadrivalent HPV vaccine.

Protection induced through anamnestic responses has been observed in the case of Hepatitis B vaccine. Like HPV, Hepatitis B is a sexually transmitted infection that, in a proportion of individuals, results in a chronic infection that leads to cancer. Like the quadrivalent HPV vaccine, the Hepatitis B vaccine is composed of the outer coat of the virus formulated on aluminium-containing adjuvant. In the case of both vaccines, the mechanism of protection is likely to be humoral, efficacy is maintained despite nominal seroreversion and anamnestic responses are induced on exposure to antigen. With these similarities in mind, the lack of breakthrough infections among subjects who became nominally anti-HPV negative may have been due to the generation of anamnestic responses leading to the clearance of early infection. Such a conclusion requires confirmation through further studies, however, given the differences between Hepatitis B infection (a blood borne infection) and HPV infection (a mucosal infection).

The present study has three limitations. First, the study had a small sample size. The duration of efficacy and the kinetics of antibody decay described herein must be confirmed. Such confirmation is being obtained through ongoing long-term follow-up of subjects who received the quadrivalent HPV vaccine in Phase III trials. Second, the antigen challenge provided in this study was given in the form of an intramuscular dose of vaccine, but the "real world" route of antigen exposure is through sexual contact. It was not possible to expose individuals to live virus, and intravaginal application of HPV L1 VLPs is a poor mimic for actual intravaginal exposure to live virions, as only the latter can induce nascent infections. Third, the study did not evaluate T cell-mediated immunity, which may play a role in the protective efficacy of these vaccines [28,29].

In summary, immune memory is the hallmark of vaccines that induce long-term protection. We have shown that a quadrivalent HPV vaccine formulated on proprietary alu-
minimum adjuvant was highly immunogenic through 5 years. The vaccine exhibited excellent immune memory as evidenced by the rapid and robust increase in vaccine-type specific antibodies in response to challenge. The duration of efficacy supports vaccination of adolescents and young adults, which is expected to greatly reduce the burden of cervical and genital cancers, precancers and genital warts.

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References