Modeling the long-term antibody response of a human papillomavirus (HPV) virus-like particle (VLP) type 16 prophylactic vaccine

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Received 26 June 2006; received in revised form 8 November 2006; accepted 15 February 2007
Available online 12 March 2007

Abstract

The duration over which antibody responses persist following HPV vaccination is unknown. To estimate the longevity of responses induced by HPV-16 vaccination, two models were fitted to serum anti-HPV-16 levels measured during a 48-month study period. The first was a conventional model of antibody decay and the second was a modified model that accounts for long-lived immune memory. Using the antibody decay model, it was estimated that following administration of a three-dose regimen of HPV-16 vaccine in women aged 16–23 years, anti-HPV-16 levels will remain above those induced naturally by HPV-16 infection for 12 years, and above detectable levels for 32 years in 50% of vaccinees. With the modified model, which fitted the data better \((p < 0.001)\), it was estimated that near life-long persistence of anti-HPV-16 following vaccination is expected at titer levels above those associated with reduction of natural HPV-16 infection in 76% of these subjects, and above detectable levels in 99% of these subjects.

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Keywords: HPV-16; VLP; Prophylactic HPV vaccine; Antibody duration; Modeling

1. Introduction

Cervical cancer is the second most common cancer in women, accounting for 250,000 deaths each year worldwide. Infection with human papillomavirus (HPV) is the first, obligate step in the development of cervical cancer \([1]\). HPV types 16 and 18 cause 70% of all cervical cancers in North America and Europe, of which HPV-16 is responsible for >50% of these cancers \([2]\).

Although cervical cancer is essentially an infectious disease \([3]\), present-day cervical cancer prevention strategies are not generally targeted towards prevention of infection. Rather, they focus on secondary prevention strategies to detect and excise dysplastic lesions before they become invasive cancer. Such approaches have reduced the rate of cervical cancer deaths by 70%, but at a significant physical and psychological cost to women, and a substantial economic and resource cost to the health care system. In developing countries, the complexity and cost of cervical cancer screening preclude widespread availability \([1,4]\).

Prevention of a disease is preferable to management of its clinical consequences. Recently, prophylactic HPV vaccines for primary prevention of cervical cancer have been evaluated in clinical studies. Such vaccines contain virus-like particles (VLPs) comprised of the L1 outer capsid protein of individual HPV types, along with immunostimulatory adjuvants \([5–10]\). Prophylactic administration of either a monovalent HPV-16
L1 VLP vaccine or quadrivalent HPV (types 6, 11, 16, 18) L1 VLP vaccine have been shown to be 100% effective in preventing the development of high-grade cervical pre-cancers and cervical cancers caused by vaccine HPV types [6,7,9]. Similarly, a bivalent HPV (types 16, 18) L1 VLP vaccine was shown to be highly effective in prevention of precancerous lesions (100%) [8,10]. Cross-protective efficacy against oncogenic HPV types 45 and 31 was also demonstrated by the bivalent vaccine [10].

Men and women are at risk for acquisition of HPV infection as long as they remain sexually active. Thus, to be effective tools for cancer prevention, prophylactic HPV vaccines must induce long-term protective efficacy. The currently available clinical trials have demonstrated that these vaccines induce robust anti-HPV responses for up to 5 years following vaccination; however, the longer-term persistence of vaccine-induced anti-HPV responses is unknown [5–11]. Furthermore, because the vaccines were highly efficacious, with no clinical evidence of waning protection, a minimum anti-HPV level associated with protective efficacy cannot as yet be defined.

While the duration of antibody response to any immunization can ultimately only be determined by long-term follow-up, several studies have mathematically modeled antibody decay following vaccination and/or natural infection for the purpose of predicting long-term immunity. When developing a model to predict long-term immunity, several dynamic factors must be considered, including rates of B-cell decay and proliferation, B-cell immune memory, cell-mediated immunity, and individual variability. Conventional models of exponential decay [12–16] and power law decay [17–19] are based upon the assumption that antibody titer will decay over time. However, these models may not be sufficient for predicting long-term immunity, as low-level antibodies can persist up to a life-time in an individual following vaccination or infection. This is attributed to ongoing production from a rapid-turnover of memory B-cell pools [20] or long-lived plasma cells [21], which can be maintained by antigenic stimulation (e.g. persistent infection, antigenic re-exposure) and/or antigen-independent activation mechanisms [22]. A few models have included parameters to account for long-term persistence, but do not account for the biological dynamics of antibody persistence and/or inter-patient variability [23–25].

With these considerations in mind, a modeling exercise was undertaken to define the long-term duration of vaccine-induced anti-HPV, based on a clinical efficacy study of an HPV-16 L1 VLP vaccine, which had been shown to be 100% effective in preventing development of HPV-16-related grade 2 or 3 cervical intraepithelial neoplasias (CIN 2/3) in women aged 16–23 years who were naïve to HPV-16 at the onset of vaccination [6,9]. The long-term duration of the antibody response induced was estimated by mathematical modeling of the antibody levels measured during a 48-month period following vaccination, using a power-law model of antibody decline based upon the biological dynamics of B-cell turnover, and a modification of this model, which additionally allows for the long-term persistence of a memory B-cell subpopulation. Although both models acceptably fitted the data and provided a range of long-term predictions, a better fit was provided by the modified model, which predicted a near life-long persistence of detectable antibodies following HPV-16 vaccination in a majority of women.

2. Methods

2.1. Study design and population

Details of the HPV-16 L1 VLP vaccine trial (Merck Research Laboratories, HPV Protocol 005) in 2409 women 16–23 years old, are described elsewhere [6,9]. Briefly, subjects were randomized to receive either HPV-16 vaccine (N = 1204) or placebo (N = 1205) at Day 1, Months 2, and 6. Subjects underwent cervicovaginal sampling for HPV infection and Pap testing at Day 1 and Months 7, 12, 18, 24, 30, 36, 42, and 48. Subjects with Pap tests suggestive of cervical dysplasia underwent colposcopy, biopsy, and definitive therapy, if clinically indicated.

Protocol 005 did not include a screening phase. Thus, subjects were enrolled regardless of baseline HPV-16 status. Among those were a group of 241 women, who at Day 1, were anti-HPV-16 seropositive, but had no evidence of ongoing cervicovaginal HPV-16 infection (HPV-16 PCR- negative). These subjects had likely been infected with HPV-16 at some time after sexual debut, and then mounted an immune response to that infection, which resulted in successful clearance of infection. Because a seroprotective level has not yet been defined for HPV-infection, subjects within this group who received placebo (n = 131) provided a reference population against which to evaluate vaccine-induced anti-HPV responses among baseline HPV-16-naïve subjects [7,9,10,28].

2.2. Follow-up study analysis

Anti-HPV-16 levels were evaluated in the vaccine and placebo groups of the per protocol immunogenicity (PPI) population (n = 1364). The PPI population included subjects who received all three vaccinations, who were HPV-16 sero- and PCR-negative at Day 1, who were PCR-negative through Month 7 (1 month Post-dose 3), and who generally did not deviate from the protocol in ways that may have impacted the immune response.

2.3. Anti-HPV-16 serologic assay

Serum samples were obtained for determination of serum anti-HPV-16 levels at enrolment, and at Months 7, 12, 18, 30, 42, and 48. Anti-HPV-16 levels were quantified using a competitive radioimmunoassay (cRIA) developed by Merck Research Laboratories, as previously described, which mea-
sured type-specific anti-HPV-16 VLP neutralizing antibody titer [23]. Results were read from a standard curve, corrected for dilution, and reported in arbitrary units (milliMerck units per milliliter, or mMU/mL). A fixed cut-off value of 5.9 mMU/mL was used to determine the serologic status of subjects [9]. Results ≤5.9 mMU/mL were reported as seronegative.

2.4. Statistical analysis of immunogenicity

Anti-HPV-16 geometric mean titers (GMTs) and the corresponding 95% confidence intervals, as well as the proportions of initially seronegative subjects achieving an HPV-16 cRIA ≥20 mMU/mL and ≥100 mMU/mL, were calculated at Months 7, 12, 18, 30, 42, and 48.

2.5. Statistical analysis and modeling of antibody duration

The persistence of anti-HPV levels over time was estimated using two mixed effects models that took into account the rates of B-cell decay. Individual antibody data measured during the 48-month study period were modeled using a conventional power law model, given by:

\[ f(t) = k - a \log (c + t) \]  (1)

where \( f(t) \) is the log antibody titer at time \( t \) post vaccination, \( k \) is the peak log level, \( a \) is the decay rate, and \( c \) is an arbitrary small constant (often set to zero). A novel derivation of this model from a mechanistic model of B-cell dynamics is presented in the appendix.

This model was then extended to account for two populations of B-cells, including activated and memory B-cells, which allows for a long-term antibody plateau given by:

\[ f(t) = k + \log [(1 - \pi)(c + t)^{-a} + \pi] \]  (2)

where \( \pi \) is the relative level of antibody produced in the long-term memory plateau (between 0 and 1). A value of \( \pi > 0 \) indicates long-term antibody persistence. Eq. (1) is recovered when \( \pi = 0 \). The derivation of this model is also given in the appendix.

The models were fitted by a mixed effects method (Nlme in SAS), where \( k \) and \( a \) are random effects, allowed to be patient-specific and are assumed to be drawn from a bivariate normal distribution. To achieve convergence of the fitting procedure, the parameter \( \pi \) was assigned as a fixed effect. This allowed a prediction of the antibody dynamics to be made for each person. Predicted results over time are reported as GMTs, and the proportion of vaccinees maintaining antibody levels above defined arbitrary thresholds.

For the power law model (1), the maximum likelihood (ML) estimates for the parameters \( k \) and \( a \) were 9.60 and 1.31. The covariance estimates were Var(\( k \)) = 2.49, Var(\( a \)) = 0.23 and Covar(\( k, a \)) = 0.58. For the modified power law model (2), the ML estimates for the parameters \( k \), \( a \), and \( \pi \) were 14.62, 3.56, and 0.000059, respectively. The covariance estimates were Var(\( k \)) = 1.43, Var(\( a \)) = 0.21, and Covar(\( k, a \)) = 0.28. In terms of long-term predictions, structural uncertainty associated with model choice is greater than statistical uncertainty in parameter estimates, hence we focused on presenting results from two differing models to bracket predictions.

2.6. Sensitivity analysis

Inter-subject variability in response to the vaccine was estimated by determining the proportions of subjects in the HPV-16 L1 VLP vaccine group with responses above various thresholds, from the study data and simulations of best-fit models. Pre-specified thresholds based upon the study protocol and results [6,9] were used: ≥5.9 mMU/mL, the lower detection limit of the serology assay; ≥20 mMU/mL, corresponding to the GMT observed in placebo-recipient women (HPV-16 seropositive and PCR-negative at Day 1), considered to have been previously exposed to natural HPV-16 infection and who mounted an immune response that presumably cleared the infection; and ≥100 mMU/mL, the GMT at the end-of-the-study (48 months), representing a conservative level five times higher than natural HPV-16 infection.

3. Results

3.1. Study population and characteristics

In Protocol 005, prophylactic administration of HPV-16 L1 VLP vaccine was 100% effective in preventing HPV-16-related cervical dysplastic lesions through a mean follow-up period of 36 months Post-dose 3 [6,9].

3.2. Anti-HPV-16 responses in the PPI population

In the placebo group within the PPI population, serum HPV-16 GMTs remained below the limits of detection (5.9 mMU/mL) throughout the duration of the study, while in the HPV-16 L1 VLP vaccine group, robust anti-HPV-16 responses were observed (Table 1 and Fig. 1). The highest anti-HPV-16 GMTs were observed at the Month 7 (1519 mMU/mL) timepoint. Anti-HPV-16 GMTs then stabilized at levels between 130 and 150 mMU/mL from Month 30 through Month 48.

At 1 month Post-dose 3, 99% and 97% of subjects who received vaccine in the PPI population had anti-HPV-16 levels ≥20 mMU/mL and ≥100 mMU/mL, respectively (Table 2). These proportions decreased to 94% and 57%, respectively, by Month 48. Only 3.3% of vaccinees who were seronegative at Month 7 became seropositive by Month 48.

The proportion of placebo recipients with titers ≥100 mMU/mL increased from 0% at Month 7 to 1.8% at Month 48, and the proportion of placebo recipients with titers ≥20 mMU/mL increased from 0.4% at Month 7 to 6.1% at Month 48. Of the placebo subjects who became seropositive
### Table 1

**Anti-HPV-16 geometric mean titers**

<table>
<thead>
<tr>
<th>Time</th>
<th>HPV-16 naive recipients (PPI population)</th>
<th>Placebo (N=1198)</th>
<th>Day 1 seropositive(^b) and PCR-negative recipients</th>
<th>Placebo (N=1198)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GMT</td>
<td>GMT</td>
<td>GMT</td>
<td>GMT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n (mMU/mL) 95% CI</td>
<td>n (mMU/mL) 95% CI</td>
<td>n (mMU/mL) 95% CI</td>
</tr>
<tr>
<td>Day 1</td>
<td></td>
<td>684 &lt;6.0 (&lt;6.0, &lt;6.0)</td>
<td>680 &lt;6.0 (&lt;6.0, &lt;6.0)</td>
<td>110 18.1 (14.1, 23.3)</td>
</tr>
<tr>
<td>Month 7</td>
<td></td>
<td>684 1518.8 (1385.5, 1665.0)</td>
<td>680 &lt;6.0 (&lt;6.0, &lt;6.0)</td>
<td>84 2552.1 (1938.2, 3360.5)</td>
</tr>
<tr>
<td>Month 12</td>
<td></td>
<td>663 369.2 (337.0, 404.5)</td>
<td>661 &lt;6.0 (&lt;6.0, &lt;6.0)</td>
<td>85 673.2 (475.0, 954.2)</td>
</tr>
<tr>
<td>Month 18</td>
<td></td>
<td>649 201.8 (184.0, 221.3)</td>
<td>638 &lt;6.0 (&lt;6.0, &lt;6.0)</td>
<td>84 534.0 (388.7, 733.5)</td>
</tr>
<tr>
<td>Month 30</td>
<td></td>
<td>609 147.4 (134.2, 161.8)</td>
<td>604 &lt;6.0 (&lt;6.0, &lt;6.0)</td>
<td>78 409.5 (295.7, 567.1)</td>
</tr>
<tr>
<td>Month 42</td>
<td></td>
<td>533 127.7 (114.1, 143.0)</td>
<td>532 &lt;6.0 (&lt;6.0, &lt;6.0)</td>
<td>72 387.0 (264.9, 565.3)</td>
</tr>
<tr>
<td>Month 48</td>
<td></td>
<td>481 131.5 (116.5, 148.4)</td>
<td>489 &lt;6.0 (&lt;6.0, &lt;6.0)</td>
<td>64 421.6 (285.2, 623.3)</td>
</tr>
</tbody>
</table>

\(N\): number of subjects randomized to the respective vaccination group who received at least one injection; \(n\): number of subjects evaluable at the given study time. CI: confidence interval; cRIA: competitive radioimmunoassay; GMT: geometric mean titer; mMU: milliMerck unit.

\(^a\) Subjects who received all three vaccinations, who were anti-HPV 16 seronegative at Day 1 and HPV-16 PCR-negative from Day 1 through Month 7, and who had no major protocol violations.

\(^b\) Anti-HPV-16 seropositive at Day 1 defined as having a Day 1 anti-HPV-16 cRIA level >5.9 mMU/mL or a positive Day 1 serum capture result.

### Table 2

**Proportion of subjects with anti-HPV-16 cRIA responses, \(\geq 20\) and \(\geq 100\) mMU/mL (PPI population)**

<table>
<thead>
<tr>
<th>Month</th>
<th>cRIA response (\geq 20) mMU/mL</th>
<th>Placebo (N=1198)</th>
<th>cRIA response (\geq 100) mMU/mL</th>
<th>Placebo (N=1198)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n Percent (%) 95% CI</td>
<td>n Percent (%) 95% CI</td>
<td>n Percent (%) 95% CI</td>
<td>n Percent (%) 95% CI</td>
</tr>
<tr>
<td>7</td>
<td>684 99.3 (98.3%, 99.8%)</td>
<td>680 0.4 (0.1%, 1.3%)</td>
<td>684 97.1 (95.5%, 98.2%)</td>
<td>680 0.0 (0.0%, 0.5%)</td>
</tr>
<tr>
<td>12</td>
<td>663 98.5 (97.2%, 99.3%)</td>
<td>661 0.8 (0.2%, 1.8%)</td>
<td>663 89.6 (87.0%, 91.8%)</td>
<td>661 0.0 (0.0%, 0.6%)</td>
</tr>
<tr>
<td>18</td>
<td>649 98.3 (97.0%, 99.2%)</td>
<td>638 1.3 (0.5%, 2.5%)</td>
<td>649 69.3 (65.6%, 72.9%)</td>
<td>638 0.3 (0.0%, 1.1%)</td>
</tr>
<tr>
<td>30</td>
<td>609 96.4 (94.6%, 97.7%)</td>
<td>604 3.8 (2.4%, 5.7%)</td>
<td>609 60.3 (56.3%, 64.2%)</td>
<td>604 0.8 (0.3%, 1.9%)</td>
</tr>
<tr>
<td>42</td>
<td>533 93.1 (90.6%, 95.1%)</td>
<td>532 4.7 (3.1%, 6.9%)</td>
<td>533 54.3 (49.9%, 58.5%)</td>
<td>532 1.1 (0.4%, 2.4%)</td>
</tr>
<tr>
<td>48</td>
<td>481 94.0 (91.5%, 95.9%)</td>
<td>489 6.1 (4.2%, 8.6%)</td>
<td>481 56.5 (52.0%, 61.0%)</td>
<td>489 1.8 (0.8%, 3.5%)</td>
</tr>
</tbody>
</table>

\(N\): number of subjects randomized to the respective vaccination group who received at least one injection; \(n\): number of subjects evaluable at the given study time. CI: confidence interval. cRIA: competitive radioimmunoassay. mMU: milliMerck units.
at any time during Months 7–48, 75% of those with anti-HPV-16 titers ≥20 mMU/mL and 82.4% of those with anti-HPV-16 titers ≥100 mMU/mL had evidence of new infection with HPV-16 as determined by PCR (data not shown). The remaining placebo subjects who became seropositive were HPV-16 PCR-negative throughout the study (25.0% and 17.6% for subjects with titers ≥20 and 100 mMU/mL, respectively).

3.3. Anti-HPV-16 response among subjects with evidence of successful clearance of HPV-16 infection prior to enrolment

In the group of placebo recipients who were seropositive and PCR-negative prior to vaccination, and who received placebo, the GMTs of placebo recipients remained near 20 mMU/mL throughout the 48-month period of the study (Table 1 and Fig. 1). In the absence of a defined seroprotective level for HPV infection, this group of placebo subjects, who were considered to have been previously infected with HPV-16 and then mounted an immune response that presumably cleared the infection, served as a reference point for the antibody response of vaccinated HPV-16 naïve subjects. Thus, it appeared that the anti-HPV GMTs induced by three doses of HPV-16 L1 VLP vaccine were at least five times greater than the GMTs generated by natural HPV-16 infection in this seropositive placebo group.

3.4. Modeling the duration of antibody protection to HPV-16 VLP vaccine

To assess the duration of the antibody response, the conventional power law model of antibody decline and the modified power law model, which considers the long-term dynamics of the antibody response, were fitted to the individual antibody data measured in the PPI population. Results from both models are presented over the first 48 months post-vaccination (Fig. 2A and B) and over 30 years post-vaccination (Fig. 2C and D). The conventional power law model predicted a continuous decline in antibody titer, while the better fitting, modified power law model (p < 0.001) predicted a decline through the first 2 years followed by a longer-term plateau (Fig. 2A). The conventional power law model estimated a median duration of detectable antibody (>5.9 mMU/mL) of 32 years; whereas the modified power law model predicted a long-term plateau of antibody duration with a near life-long persistence above the level of detection (>5.9 mMU/mL).

3.5. Proportion of subjects in the PPI population with detectable anti-HPV as estimated by the models

To assess the robustness of the models in terms of the inter-subject variability, the proportion of subjects with responses above various thresholds was estimated (Fig. 2C and D) and observationally compared to the actual study data through 48 months. Although the minimum protective antibody level for HPV has not yet been defined, threshold levels of 20 and 100 mMU/mL were chosen on the basis of anti-HPV-16 levels induced by HPV-16 infection and vaccination, respectively (Table 1), which are somewhat higher than the detection limit threshold of 5.9 mMU/mL.

The proportions of vaccinees who will maintain anti-HPV-16 levels above these thresholds as a function of time were predicted using both models (Fig. 2C and D). Both models predicted that >50% of vaccinees will maintain anti-HPV-16 levels above all three thresholds during the 48-month study period (Fig. 2C), which was similar to the observed data in the study (Table 2). Fig. 2A and C show that the modified power model provide the better fit to the data. The conventional power law model predicted that at 32 years, 50% of the vaccinees will have anti-HPV-16 levels above detectable levels (>5.9 mMU/mL); whereas the modified model predicted that 99% of the vaccinees will experience near life-long detectable anti-HPV-16 levels (>5.9 mMU/mL) (Fig. 2D). Additionally, the power law model estimated that anti-HPV-16 levels will remain above levels induced by HPV-16 infection (>20 mMU/mL) for 12 years in 50% of vaccinees who received the HPV-16 L1 VLP vaccine, and the modified model estimated that 76% of vaccinees will maintain life-long anti-HPV-16 levels above those induced by HPV-16 infection (>20 mMU/mL) (Fig. 2D).

3.6. Distribution of anti-HPV-16 level and correlation of peak and plateau levels predicted by the modified model

The distributions of the immediate Post-dose 3 (Month 7) and long-term plateau of anti-HPV-16 titers estimated from the better-fitting modified model were similar to the distributions of the observed immediate Post-dose 3 (Month 7) and end-of-study titers at 48 months (Fig. 3A and C). The correlation of the peak and end-of-study anti-HPV-16 levels was
Fig. 2. Model-based prediction of GMTs and proportions above different thresholds following HPV-16 L1 VLP vaccination predicted from the models. GMTs predicted from the power-law (- - -) and modified power law (—) models, using antibody data measured during 48 months following HPV-16 L1 VLP vaccination, are shown in (A) for 48 months and in (B) for 30 years. GMT data points measured in the study at indicated times (♦) are shown in (A). Proportions above the different thresholds predicted from the power-law (- - -) and modified power law (—) models, using antibody data measured during 48 months following HPV-16 L1 VLP vaccination, are shown in (C) for 48 months and in (D) for 30 years. Proportions of vaccines measured in the study above 100 (♦), 20 (△), and 10 (□) mMU/mL are shown in (C). Since model uncertainty is greater than statistical uncertainty, the two models bracket long-term predictions; however, of the two, the modified model fits the data significantly better.

Fig. 3. Distribution and correlation of peak and end-of-study titers. (A) The distribution of peak (Month 7) and plateau antibody levels based on 10,000 simulations from the best-fit mixed effects model. (B) The correlation between the peak (Month 7) and plateau (Month 48) titers is illustrated for 250 simulations from the best-fit mixed effects model. (C) The distribution of peak (Month 7) titer and end-of-study (Month 48) titers measured in the study. (D) The correlation between the peak (Month 7) and end-of-study titers (Month 48) observed in the study.
$R^2 = 0.518$ for the modified model, and that of the study for the observed peak and end-of-study titers was $R^2 = 0.192$ (Fig. 3B and D). While both the model and the study showed positive correlations, the lower correlation for the study reflected outliers and a degree of longitudinal variability that was not fully captured by the model.

4. Discussion

An ideal HPV prophylactic vaccine would provide protection for at least 10–15 years during the greatest risk period for HPV infection [27]. In this study, mathematical modeling of the long-term anti-HPV-16 responses following vaccination with an efficacious HPV-16 L1 VLP vaccine [6,9] provided estimates of the duration of detection of vaccine-induced anti-HPV-16 ranging from 12 years to near life-long persistence in a majority of women, 16–23 years of age.

In Protocol 005, administration of HPV-16 L1 VLP vaccine to baseline HPV-16-naïve women was highly effective in preventing development of HPV-16-related cervical precancers through the 48-month study period. Anti-HPV-16 GMTs among baseline HPV-16-naïve women who received the full vaccine regimen were at least five-fold higher than the anti-HPV-16 GMTs of a group of placebo recipients in the study who were considered to have previously encountered and cleared an HPV-16 infection (seropositive and PCR-negative) prior to vaccination and who received placebo [8]. The anti-HPV-16 GMTs of these previously-infected individuals were stable at a low level throughout the 48-month study period, reflecting the durability of antibody responses following HPV-16 infection. This was also reflected by the proportion of naïve recipients in the placebo group who became HPV-16 seropositive during the 48 months in the study, the majority of whom experienced new HPV-16 infections. Furthermore, repeated exposure of HPV-seropositive individuals to the same HPV types appear to result in an anamnestic response which may also contribute to the long-term persistence of anti-HPV responses [9,28]. Potentially a low level of exposure to cross-reactive HPV-types may also stimulate immune memory [10,21]. Taken together, these data suggested that the persistence of anti-HPV-16 antibodies induced by vaccination and/or HPV-16 infection may be long-lasting.

The long-term persistence of the anti-HPV-16 response was estimated using a model of antibody decline which accounted for the kinetics of B-cell turn-over [17–19], and a modified model, which was developed to additionally allow for the long-term persistence of a memory B-cell subpopulation and a long-lived antibody plateau. Notably, the modified model provided a better fit of the data ($p < 0.001$), indicating the importance of accounting for long-term antibody memory, and was consistent with the stable and durable immune responses induced by HPV-16 vaccination in this study. However, in the absence of additional follow-up data, the possibility of immunosenescence and/or a second phase of very slow antibody decline could not be assessed and therefore cannot be ruled out (see Appendix A).

In addition to modeling the antibody duration based on the mean anti-HPV-16 titers induced by vaccination, the variability of the antibody responses was characterized. Although an anti-HPV-16 level associated with protective efficacy has not been defined, variability was assessed by estimating the proportion of vaccinees with anti-HPV-16 responses above the assay limit of detection, HPV-16 infection-induced levels, and vaccine-induced levels at the end of the study. Both models adequately described the proportions of antibody responses above these thresholds (Fig. 2); however, the better fitting modified model returned estimates more similar to those observed in the study, as well as comparable distributions of peak and plateau titers (Fig. 3). This further supported the suitability of the modified model in predicting anti-HPV-16 persistence in this study.

The suitability of the modified, longer-term model may be attributed to the inclusion of parameters that account for the dynamics of the humoral response to antigen stimulation. When compared with models previously used to estimate antibody persistence following vaccination, this model provided assumptions for the contribution of B-cell decay and differentiation, and memory B-cells in addition to antibody decay [21,29], as well as accounting for inter-subject variability.

The comparability of vaccine-induced anti-HPV-16 responses and natural immune responses induced by HPV-16 infection remains to be determined. The immune response to HPV infection is characterized by a localized cell-mediated response associated with lesion regression and the generation of low levels of neutralizing antibodies in most individuals, resulting in the clearance of infection [26]. Little is known about the persistence of antibody and seroprotective effect following HPV infection; however, the existing data suggest that a prolonged antigen exposure is required for a robust antibody response and long-term persistence has been shown to correlate with large antibody responses [31–34].

The estimates of antibody duration in this study were based upon titers of type-specific antibodies that recognize neutralizing epitopes on HPV VLPs. Although these titers were considerably higher than the antibody levels induced by HPV-16 natural infection, even substantially greater titers of total anti-VLP antibodies (27- to 938-fold) have been shown to be induced by HPV-16 VLP vaccination compared to natural infection levels [10,35]. While type-specific antibodies to VLP neutralizing epitopes may be associated with conferring anti-HPV-16 efficacy, these antibodies comprise only a portion of the total anti-VLP response; hence the prediction of antibody duration in this study may be a conservative approach.

Both T- and B-cell responses are required in the establishment of immune memory. It is likely that T-cell responses contribute to the long-term antibody persistence stimulated by a three-dose regimen of HPV-16 L1 VLP vaccine, and may also be accounted for in our long-term modified model.
T-cells play a role in the achievement of long-lasting neutralizing antibody levels by promoting the generation and maintenance of B-cell responses [29]. Both helper and cytotoxic T-cell responses are induced by HPV-16 L1 VLPs [36], however, the biological relevance of these responses to natural disease and vaccination has not been demonstrated. Helper and cytotoxic T-cell responses have also been demonstrated following natural HPV infection, although the role of these cells in natural infection is not yet understood [30,37].

Ultimately, unequivocal determination of the long-term efficacy of HPV L1 VLP vaccines and the persistence of the anti-HPV responses induced by these vaccines can only be ascertained empirically by long-term follow-up analyses. Further work is needed to define antibody levels associated with protection [38], a difficult undertaking while efficacy remains uniformly high following vaccination [6,7,9–11]. The long-term estimates of antibody persistence predicted in this study are reasonable based upon the long-lasting, anti-HPV-16 responses demonstrated during the 48-month study period. However, it is possible that for some individuals, administration of booster doses may be required for full protection through the 10–15 years risk period.

The model developed herein may be applicable to the long-term estimation of antibody persistence for other vaccines, valuable to the establishment of practice guidelines for future vaccines. However, its applicability awaits validation using antibody responses from another vaccine with longer-term follow-up data. Importantly, while this study indicates the potential for long-term anti-HPV-16 protection during the highest risk period for HPV infection in women, the antibody duration of the recently approved quadrivalent vaccine Gardasil which includes HPV types 6, 11, 16, 18 must be assessed. HPV types do not appear to interfere with antibody response [28], thus, it is anticipated that the presence of additional anti-HPV types will not influence the model predictions by interference. Furthermore, there is evidence of cross-protection among HPV types [10] following HPV L1 VLP vaccination which may enhance immune memory, and can be accounted for by the parameters in this model.

Appendix A. Derivation of the models for antibody dynamics

We derive the model of antibody dynamics in several stages, for the purpose of clarity of exposition. By developing a novel derivation of the conventional power-law model of antibody decay, we obtain a natural framework in which to extend the model to allow for long-term persistence.

A.1. Simple exponential decay

Consider a single class of B-cells decaying at constant rate $a$, producing antibody at rate $B$, which itself decays at rate $d$. Once the antigenic stimulus has passed and B-cell proliferation has ended, the quantity of B-cells, which we denote $X(t)$, and the quantity of antibody, which we have denoted $F(t)$, will be governed by the following equations:

\[
\frac{dX}{dt} = -aX \\
\frac{dF}{dt} = BX - dF
\]  

(A.1)

The rate of decay of free antibody ($d$) is much faster than the rate of decay of B-cells ($a$), so the ratio of free antibody to B-cells will be approximately constant, given by $F(t) \approx BX(t)/d$. Eq. (A.1) reduces to:

\[
\frac{dF}{dt} = -aF
\]  

(A.2)

The solution to this model is simple exponential decay $F(t) = K \exp(-at)$. The exponential model of decay has not been found to describe post-challenge antibody dynamics well.

A.2. Conventional power-law decay

There may be some heterogeneity in the rate of decay of B-cells. Models where antibody titer decays as a negative power of time have been conventionally used to model antibody dynamics [14–16], and can be obtained by modifying the model above so that the decay rate is given by a Gamma distribution. Consider this with mean $m$ and coefficient of variation $k$ (=mean/variance), given by

\[
p(x) = \frac{k(kx)^{mk-1} \exp(-kx)}{\Gamma(mk)}
\]  

(A.3)

where $x$ is the decay rate and $p(x)$ is its probability distribution. To obtain the overall decay curve for the antibody concentration, one integrates overall possible decay rates $x$, i.e.

\[
F(t) = K \int_0^\infty p(x) \exp(-xt) dx = K \left(1 + \frac{t}{k}\right)^{-mk}
\]  

(A.4)

This is easily reparameterized into equation [1] for describing the log-antibody titer.

A.3. Long-term antibody persistence

We can now extend this model by considering two distinct populations of B-cells, designated active and memory. In the first instance, we can assume that the memory population does not decay (as may be plausible if it is continuously renewed), and makes up a proportion $\pi$ of the cell population at the peak of the response. The function for antibody dynamics is

\[
p(x) = (1 - \pi) \frac{k(kx)^{mk-1} \exp(-kx)}{\Gamma(mk)} + \pi
\]  

(A.5)

Integrating this as in Eq. (A.4) and reparameterizing as above yields the proposed model equation [2].
A.4. Slow second phase of antibody decay

As a final possibility, we considered that the memory cell population could itself decay at mean rate $\nu$ with the same coefficient of variation, so that the decay curve becomes

$$F(t) = K \left[ (1 - \pi) \left( 1 + \frac{t}{k} \right)^{-\mu k} + \pi \left( 1 + \frac{t}{k} \right)^{-\nu k} \right] \quad (A.6)$$

We found, however, that we had insufficient data to estimate the parameter $\nu$. Our approach can thus be thought of as a consideration of the bounds on $\nu$, namely $0 \leq \nu \leq \mu$.

References


