Vaccination with multimeric L2 fusion protein and L1 VLP or capsomeres to broaden protection against HPV infection

Subhashini Jagu a, Kihyuck Kwak a, Robert L. Garcea b, Richard B.S. Rodena,c,d,∗

a Department of Pathology, Johns Hopkins University, Baltimore, MD 21231, USA
b Department of Molecular, Cellular, and Developmental Biology, University of Colorado, 347UCB Boulder, CO 80309, USA
c Department of Oncology, Johns Hopkins University, Baltimore, MD 21231, USA
d Department of Gynecology and Obstetrics, Johns Hopkins University, Baltimore, MD 21231, USA

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Immunization with L1 as pentavalent capsomeres or virus-like particles (VLPs) generates high and long-lived titers of neutralizing antibodies and protection primarily against the human papillomavirus (HPV) type from which the vaccine was derived. Conversely, vaccination with L2 minor capsid protein derived from multiple HPV types induces lower titer, but more broadly neutralizing and protective antibody responses. We combined the advantages of each protective antigen by immunization with titrated doses of multi-type L2 with either L1 capsomeres or VLP. We observed no significant interference between the L1 and L2 antibody response upon co-administration of L1 vaccines with multi-type L2 vaccines.

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1. Introduction

HPV is responsible for 5% of all cancers worldwide, including cervical cancer and the majority of vaginal, vulvar, penile, anal and a subset of certain head and neck cancers [1]. Although HPV16 and HPV18 cause 50% and 20% of cervical cancers respectively, there are more than a dozen other ‘oncogenic’ types of genital HPV [2]. Long-term protection against all oncogenic types through vaccination is necessary to eventually eliminate cervical cancer and the need for expensive screening programs [3–5]. Other benign HPV types are responsible for considerable morbidity, including genital warts associated primarily with HPV6 and HPV11 infections. The currently licensed vaccines, Cervarix and Gardasil, are derived from major capsid protein L1 virus-like particles self-assembled in insect or yeast cells respectively [6,7]. These vaccines both target the two most important oncogenic HPV types, HPV16 and HPV18, although Gardasil also contains HPV6 and HPV11 L1 VLP to protect against benign genital warts. The current vaccines do not target benign cutaneous warts associated with numerous types including HPV1 or the plethora of epidermodysplasia verruciformis (EV)-associated HPV types, such as HPV5, linked to non-melanoma skin cancers that afflict the genetically-predisposed EV patients and immunocompromised individuals in particular [8].

Immunization with L1 VLPs generates high titer serum neutralizing antibodies that are primarily type-specific, although limited cross-reactivity with the additional oncogenic types associated with cervical cancer has been observed [9,10]. L1 VLP are protective even without an adjuvant [11–14], but the current vaccines both are formulated in aluminum salts (amorphous aluminum hydroxyphosphate sulfate in Gardasil and aluminum hydroxide in Cervarix) and Cervarix also includes the TLR4 agonist monophosphoryl lipid A (MPL), presumably with the goal of enhancing cross-neutralization of closely related types and sustaining the neutralizing antibody response [3]. The licensed L1 VLP vaccines provide near complete protection from the HPV types from which they are derived and the limited in vitro cross-neutralization detected is somewhat predictive of partial activity against other highly phylogenetically-related types [15]. In the case of Cervarix, vaccination provides strong protection against HPV31 and HPV45, the two types most closely related to HPV16 L1 and HPV18 L1 respectively from which its constituent VLPs were generated [16]. However, the relative neutralization titers are much lower for heterologous types compared with the homologous type, and
therefore the longevity of this cross-protection is uncertain [10,17]. Further, protection against most other oncogenic types is limited, and no protection is provided against other benign HPV infections presumably [16]. The breadth of protection might be enhanced by increasing the valency of current HPV vaccines, and efforts to produce eight or nine type L1 VLP vaccines are ongoing. Nonetheless, gaps in the coverage might remain, and this approach greatly increases the complexity of the manufacture and trials and therefore likely the cost of vaccination. Cost is a critical issue for the worldwide introduction of HPV vaccination, and the breadth of protection is particularly significant for countries lacking cytologic screening programs [18]. Since the licensed HPV vaccines do not protect against all oncogenic HPV types, the cost of vaccination must currently be borne in addition to the continued cytologic screening programs, reducing the cost benefit. Another unintended consequence is that the predictive value and cost effectiveness of current screening regimens plummets in vaccinated women [18].

L1 capsomers also induce high titers of neutralizing antibodies, but unlike the eukaryotically-expressed VLPs in the licensed vaccines, they are produced at high level in Escherichia coli and represent a potential low cost alternative [19–21]. While the administration of low doses of canine oral papillomavirus (COPV) L1 capsomers as glutathione-S-transferase (GST) fusions and without adjuvant, as described earlier for COPV L1 VLP, provides naive dogs complete protection from experimental viral challenge [21], they may be similarly or less immunogenic than L1 VLP depending on the construct [22–25]. Notably the HPV16 L1Δ10 capsomers exhibited comparable immunogenicity to L1 VLPs [24,25]. However, the immunogenicity of L1 capsomers has not been directly compared to a licensed HPV vaccine using the same adjuvant system. Since passive transfer of naïve animals with L1 VLP-specific serum IgG provides protection, neutralizing antibodies are the relevant immune correlate of protection [11,12]. Thus the relative ability of L1 capsomers to induce a non-neutralizing antibody response to the licensed HPV vaccines is an important issue for their clinical development.

Vaccination with the minor capsid protein L2 also protects animals from papillomavirus challenge by the induction of neutralizing antibodies, albeit at much lower titers than induced by L1 VLP [26–31]. Interestingly, vaccination with HPV16 L2 protects rabbits from challenge with cotton tail rabbit papillomavirus (CRPV), suggesting, in contrast to L1 VLP, that L2 vaccination might provide broad immunity to diverse HPV types. L2 contains widely cross-neutralizing epitopes that are presumably conserved because they contribute to some critical viral function such as infectivity [30,32]. Unfortunately L2 is poorly immunogenic compared to L1 VLP, and it is therefore unclear how long immunity could be maintained [33]. The immunogenicity of L2 might be improved by vaccination at higher doses and/or formulation in potent adjuvants, such as alum in combination with a TLR9 agonist like ISS 1018 [34,35], or a TLR4 agonist like monophosphoryl lipid A (MLP) that provide a ‘danger signal’ [36,37]. While L2 residues 11–88 contain cross-protective epitopes, it is clear that L2-specific antibodies typically neutralize related types more efficiently than less evolutionarily related types. To enhance cross-protection by reinforcing the common epitopes, we designed multi-type L2 fusion proteins consisting of known cross-neutralizing epitopes of divergent HPV types. For example, polypeptides comprising residues 11–88 from the L2 genes of HPV1, HPV5, HPV6, HPV16 and HPV18 L2 were concatenated to form the multi-type L2 11–88 × 5 antigen and produced in the low cost E. coli expression system [36,38].

Here our objective was to foster development a broadly protective HPV vaccine that might be produced inexpensively to meet the needs of low resource countries by combining vaccine antigens and several adjuvants. Specifically we examined whether higher doses and formulation of the L2 11–88 × 5 vaccine in alum and TLR agonists could enhance the sustained neutralizing antibody response against diverse oncogenic HPV types, and whether combining the L2 11–88 × 5 vaccine with Cervarix or HPV16 L1 capsomers could enhance the breadth, strength and longevity of the neutralizing antibody response as an alternative to the generation of highly multivalent L1 vaccines.

2. Materials and methods

2.1. L2 11–88 × 5 antigen preparation

The 11–88 regions of L2 derived from HPV 1, HPV5, HPV6, HPV16 and HPV18 were codon optimized for E. coli expression by lowest free energy calculation and synthesized fused in frame by Blue Heron Inc. with 5’ BamHI and 3’ XhoI sites to facilitate subcloning [36]. The L2 11–88 × 5 gene was cloned into the pET28a vector (Novagen) and hexahistidine-tagged recombinant polypeptides expressed in E. coli BL21 (Rosetta cells, Novagen) [32]. The hexahistidine-tagged recombinant L2 polypeptide 11–88 × 5 was affinity purified by binding to a nickel-nitriotriacetic acid (Ni-NTA) column (Qiagen) in 8 M urea (using the QiAExpressist standard purification protocol for denaturing conditions) and then dialyzed in cassettes (Pierce) against Dulbecco’s phosphate buffered saline (PBS). Purity was monitored by SDS-PAGE and protein concentration determined by bicinchoninic acid test (Pierce) using a bovine serum albumen standard [36]. Endotoxin levels were tested using Limulus Amebocyte Lysate (LAL) QCL-1000 (Lonza Walkersville Inc., Walkersville, MD, USA). The HPV antigens were adsorbed on to aluminum hydroxide (50 μg Al3+ as Al(OH)3, Sigma) alone or in the presence of 5 μg of Monophosphoryl lipid A (MPL, Sigma) or 10 μg ISS 1018 (Dynavax, Berkeley, CA) overnight at 4 °C rotating end over end. In the case of mixing Cervarix and L2 11–88 × 5, the L2 11–88 × 5 protein was added to Cervarix (GSK) and incubated overnight at 4 °C rotating end over end.

2.2. Preparation of GST-HPV16 L1 capsomers

Full length HPV16 L1 was expressed as a GST fusion protein in BL21 (DE3) Codon Plus E. coli (Stratagene) using previously described conditions for protein induction and bacterial growth [20,39,40]. The fusion protein was purified as previously described [39] with the following modifications. Lysozyme (500 μg/ml) and sodium deoxycholate (0.1% final) were added to the cells in buffer L (50 mM Tris–HCl, pH 8.0, 0.2 M NaCl, 1 mM DTT) prior to sonication. DNAase I was added to the lysed bacteria at a concentration of 40 μg/ml, along with 2 mM ATP and 10 mM MgCl2. After incubation for 2 h at 4 °C, solid urea was added to a final concentration of 2.3 M along with PMSF, leupeptin and pepstatin A protease inhibitors. After a further incubation for 2 h at RT, the lysate was dialyzed overnight into three changes of buffer without urea. After centrifugation of the dialyzed lysate at 25,000 × g for 75 min, the clarified lysate was applied to a glutathione sepharose column. The column was washed with 4 column volumes each of buffer L, buffer L plus 5 mM ATP and 5 mM MgCl2, buffer L plus 2.5 M urea, and buffer L, before elution with buffer L with 10 mM reduced glutathione. SDS-PAGE analysis of the eluted fusion protein revealed GST-L1 and free GST as the major components. Total GST-L1 protein in the sample used for vaccination was estimated by SDS-PAGE in comparison to a BSA standard.

2.3. Enzyme-linked immunosorbent assays (ELISAs)

Immobilin plates (Nunc) were coated with 100 ng/well of 6His-11–88 × 5 L2 (prepared in E. coli) or HPV16 L1 VLPs (produced in 293TT) cells in PBS overnight at 4 °C. Wells were then blocked with 1% bovine serum albumin (BSA)–PBS for 1 h at room temperature,
and incubated with 2-fold dilutions of mouse sera for 1 h at room temperature. Following a wash step with PBS-0.01% (v/v) Tween 20, peroxidase-labeled rabbit anti-mouse (Amersham) diluted 1:5000 in 1% BSA–PBS was added for 1 h. The plates were then washed and developed with 2.2’-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid solution (Roche) for 10 min.

2.4. Neutralization assays

The papillomavirus pseudovirion in vitro neutralization assays were performed as described earlier [36,41] and the secreted alkaline phosphatase content in the clarified supernatant was determined using the p-Nitrophenyl phosphate tablets (Sigma, St. Louis, MO) dissolved in diethanolamine and absorbance measured at 405 nm. Constructs and detailed protocols for the preparation of the pseudovirions can be found at http://home.ccr.cancer.gov/1co/.

Titers were defined as the reciprocal of the highest dilution that caused a 50% reduction in 

2.5. Animal studies

Studies were performed with the approval of the Johns Hopkins University Animal Care and Use Committee. Balb/c mice (NCI Frederick) were vaccinated in groups of 5 animals three times at 2-week intervals s.c. with different concentrations of L2 11–88 × 5 alum alone, or with Alum + MPL, alum + ISS 1018 or with 1/10th of human dose of Cervarix (comprising 2 μg of HPV16 L1 VLP and 2 μg of HPV18 L1 adsorbed on to aluminum hydroxide (50 μg Al3+ as Al(OH)3) and 5 μg of monophosphoryl lipid A) or 2 μg of GST-HPV16L1 capsomers in alum + MPL. Serum samples were obtained by tail vein bleeds 2 weeks and 4 months after the final immunization.

2.6. Statistical analysis

Comparison of Log10 transformed titers by ANOVA with Bonferroni correction for multiple comparisons and graphical presentation of titers as box/whisker plots were performed using GraphPad Prism 4 software. Significance was set at p < 0.05. For samples lacking reactivity at the lowest serum dilution, a titer of 50 was not considered significant.

3. Results

3.1. Impact of adjuvant on the humoral response to L2 11–88 × 5 vaccination

We have previously shown that vaccination of mice with a multimeric L2 protein formulated in alum and the TLR9 agonist 1018 ISS (CpG) induces long-lived protection and broadly neutralizing serum antibodies [36]. Nevertheless, the titers of neutralizing antibodies induced by the multimeric L2 construct were lower than those elicited by L1 VLP, and therefore we have explored alternative adjuvants to boost the L2-specific neutralizing antibody titers. Since TLR9 activates only the MyD88 pathway, but TLR4 activates both the MyD88 and Trif-dependent innate signaling pathways [37,42], here we have explored the adjuvant activity of alum in combination with the TLR4 agonist MPL for L2-specific humoral responses. To assess the ability of each formulation to adjuvant L2-specific responses we examined dose sparing, peak neutralizing antibody titers and long-term responses for both HPV types used to derive the L2 11–88 × 5 vaccine (herein termed ‘homologous’) and types whose L2 was not represented in the vaccine (termed ‘heterologous’). As the most stringent model, we utilized mice for vaccination since prior studies indicate that L2-specific immune responses are less robust in mice as compared to rabbits for this immunogen [36]. Therefore, Balb/c mice were vaccinated three times at 2-week intervals with decreasing doses of L2 11–88 × 5 (20, 10, 4, 2, 1, or 0 μg) in alum, alum + 10 μg 1018 ISS (CpG), or alum + 5 μg MPL and 2 weeks later a serum sample was harvested. The L2 11–88 × 5-specific humoral immune response was first analyzed by ELISA using the L2 immunogen to coat the microtiter plate (Fig. 1). Mice vaccinated with adjuvant alone failed to elicit an L2-specific antibody response. Conversely mice vaccinated with L2 11–88 × 5 in each of the formulations generated high titer serum antibody responses against the antigen, and a dose response was apparent. The significant dose response for the peak ELISA titers (p < 0.001) in mice vaccinated with L2 11–88 × 5 in alum was not significantly different to the alum + MPL formulation of L2 11–88 × 5, whereas the titers were significantly higher in antisera of mice receiving the lower doses of L2 11–88 × 5 formulated in alum + CpG (p < 0.001 at the 1 μg dose). L2 ELISA titers and L2-specific neutralizing antibody titers tend to correlate less well with each other than L1 VLP ELISA titers and L1-specific neutralization titers, probably because its neutralizing epitopes comprise a small fraction of the entire L2. Therefore, we examined the titers for in vitro neutralization of HPV16, a type whose L2 is present within the 11–88 × 5 immunogen (Fig. 2A). The L2 11–88 × 5 formulation in alum exhibited a significant dose response between 1 and 20 μg (p = 0.008). No significant difference between the dose response curves for the peak HPV16 neutralization titers was observed for L2 11–88 × 5 formulated in the three adjuvants. However, when we examined the in vitro neutralization titers for HPV18 and for HPV45, a type whose L2 was not utilized to produce the L2 11–88 × 5 antigen, the alum + CpG and alum + MPL formulations appeared to be more effective in triggering detectable titers with low doses of antigen (Fig. 2B and C, see 2 μg dose).

Adjuvants can increase the long-lived or ‘set point’ titers and therefore we compared the three formulations for their ability to maintain detectable antigen-specific serum titers. At 4 months after immunization serum samples were again harvested from each mouse and tested for HPV16 neutralization titer (Fig. 3A). No sig-
Fig. 2. Influence of adjuvant upon L2-specific neutralizing antibody titers. Sera from each mouse was collected 2 weeks after the third dose of the indicated vaccines was titrated in 2-fold dilutions from 1:50 and tested for *in vitro* neutralization of HPV16 (A), or HPV18 (B), or HPV45 (C) pseudovirions.

Fig. 3. Influence of adjuvant upon L2-specific neutralizing antibody titers 4 months after vaccination. Sera from each mouse was collected 4 months after the third dose of the indicated vaccines was titrated in 2-fold dilutions from 1:50 and tested for *in vitro* neutralization of HPV16 (A), or HPV45 (B), or HPV58 (C) pseudovirions.
significant difference was observed for the dose response curves for L2 11–88 × 5 formulated in the three adjuvants. However, when examining the neutralization of HPV45, the titers were trending higher at the lowest doses of antigen for the alum + MPL formulation as compared to the alum alone or alum + CpG formulations, but this did not reach significance (Fig. 3B). To expand this analysis of heterologous neutralization, we also examined the set point titers for HPV58, another type whose L2 sequences were not used to generate the L2 11–88 polypeptide. Again the in vitro neutralization titers in the alum + MPL group exhibited a trend for superiority as compared to the alum alone or alum + CpG formulations (Fig. 3C).

3.2. Combination of the L2 11–88 polypeptide compared to the alum alone or alum + CpG formulations (Fig. 3C). To expand this analysis of heterologous neutralization, we also examined the set point titers for HPV58, another type whose L2 sequences were not used to generate the L2 11–88 polypeptide. Again the in vitro neutralization titers in the alum + MPL group exhibited a trend for superiority as compared to the alum alone or alum + CpG formulations (Fig. 3C).

3.2. Combination of the L2 11–88 × 5 vaccine with L1

One possible approach to provide the broadest possible immunity against oncogenic HPV infection is to mix the L1 VLP vaccine with a multimeric L2 polypeptide. While it is possible that the ability of VLP to activate dendritic cells may adjuvant the humoral responses to the multimeric L2 component [43,44], it is also possible that the L1-specific response so dominates that the L2-specific antibody titers in the mice receiving Cervarix alone, capsomeres alone or adjuvant (alum + MPL) alone. Conversely, the antisera of mice receiving L1 capsomeres and L2 11–88 × 5 did not impact the HPV16 neutralizing antibody titer. Vaccination with 2 μg of HPV16 L1 VLP in Cervarix as compared with 2 μg of HPV16 L1 capsomeres in an equivalent adjuvant (p < 0.001). Importantly, the HPV16 L1 VLP-specific ELISA titers to either Cervarix or L1 capsomeres were unaffected by co-administration of up to 20 μg of L2 11–88 × 5, approximately 10 times the amount of L1 in this formulation.

Examination of the HPV16 in vitro neutralization titers of the antisera also showed a 10-fold higher titer in mice receiving Cervarix as compared with L1 capsomeres (p < 0.01, Fig. 5A). While this may be important for clinical development based upon the induction of non-inferior neutralizing antibody titers, the immune response to GST-L1 capsomeres is highly protective in animal models [21], and it is clear from the cross-protection seen with the licensed HPV vaccines that extremely low serum neutralizing antibody titers to non-vaccine types are protective [15,16].

The HPV16 in vitro neutralization titers of mice vaccinated with L2 11–88 × 5 were 10-fold lower than for 2 μg of HPV16 L1 capsomeres, even when 20 μg of L2 11–88 × 5 was administered (p < 0.001, Fig. 5A). Vaccination with Cervarix in the presence of L2 11–88 × 5 did not impact the HPV16 neutralizing antibody titer. Vaccination with 2 μg of L1 capsomeres mixed with 10 or 20 μg of L2 11–88 × 5 exhibited a trend for a slightly higher titer, suggestive of an additive effect such that these titers were no longer significantly different from Cervarix alone.

Vaccination of mice with Cervarix alone or with the L2 11–88 × 5 resulted in similarly high HPV18 neutralizing serum antibody titers, as seen for HPV16 (Fig. 5B). No HPV18 neutralizing antibody titers were detected in the sera of mice vaccinated with HPV16 L1 capsomeres, whereas the mice vaccinated with L2 11–88 × 5 contained robust titers of HPV18 neutralizing antibodies, albeit ~1000-fold lower than observed for Cervarix vaccinated animals (p < 0.001).

There were no detectable titers for neutralization of HPV35, HPV45 and HPV58 in the antisera of mice vaccinated with adjuvant alone, or L1 capsomeres or even Cervarix alone (Fig. 5C–E). Conversely, the antisera of mice receiving L1 capsomeres and L2

![Fig. 4. L1 VLP and L2-specific antibody titers. Sera from each mouse was collected 2 weeks after the third dose of the indicated vaccine antigens in alum + MPL was titrated in 2-fold dilutions from 1:50 and tested by ELISA using plates coated with L2 11–88 × 5 (A), or HPV16 L1 VLP (B).](image)
Influence of combining L2 11–88 × 5 with L1-based HPV vaccines upon neutralizing antibody titers. Sera from each mouse was collected 2 weeks after the third dose of the indicated vaccine antigens in alum + MPL was titrated in 2-fold dilutions from 1:50 and tested for \textit{in vitro} neutralization of HPV16 (A), HPV18 (B), HPV35 (C), HPV45 (D) or HPV58 (E) pseudovirions.

11–88 × 5 were able to effectively neutralize HPV35, HPV45 and HPV58, none of which was used to derive the L2 11–88 × 5 vaccine (Fig. 5C–E). While the antisera of mice receiving L1 capsomeres and L2 11–88 × 5 exhibited similar titers of HPV35, HPV45 and HPV58 neutralization as mice receiving L2 11–88 × 5 alone, there was a trend for reduced titers in antisera of mice vaccinated with Cervarix and L2 11–88 × 5. Indeed, we failed to detect HPV45 or HPV58 neutralizing antibodies in several mice vaccinated with Cervarix and L2 11–88 × 5.

Finally, we examined the impact of mixing L2 11–88 × 5 with Cervarix or L1 capsomeres upon the \textit{in vitro} neutralizing antibody titers at 4 months post-vaccination (Fig. 6). The trends in the \textit{in vitro} neutralization titers observed at 2 weeks after vaccination and described above were preserved at 4 months and the titers remained stable between 2 weeks and 4 months for each antigen (Figs. 5 and 6).

4. Discussion

This study suggests that the formulation of an L2 multimeric construct with both alum and MPL or CpG may be beneficial over alum alone based upon increased \textit{in vitro} neutralization titers for
Fig. 6. Influence of combining L2 11–88 × 5 with L1-based HPV vaccines upon neutralizing antibody titers at 4 months after vaccination. Sera from each mouse was collected 4 months after the third dose of the indicated vaccine antigens in alum + MPL was titrated in 2-fold dilutions and tested for in vitro neutralization of HPV16 (A), or HPV45 (B), or HPV58 (C) pseudovirions.

HPV types not used to construct the antigen, particularly for the long-term immunity. It is important to recognize that humans may exhibit a distinct response to mice, but the addition of MPL or CpG can boost the antibody titers in comparison to VLP in alum alone [34,45]. Another important caveat is that the L2 11–88 × 5 was not endotoxin-free, but contained as much as 25 EU in the 20 μg formulation (and corresponding less at the lower doses). While the 5 μg of MPL added in vaccine formulation corresponds to >2500 EU, the endotoxin present in the L2 11–88 × 5 preparation may have masked the differences between the adjuvant formulations. Notably in an earlier study we observed a reduced degree of protection of mice from cutaneous challenge with HPV16 pseudovirions at 4 months after vaccination with another L2 multimeric polypeptide (L2 11–200 × 3) formulated in alum alone versus alum + ISS 1018 [36].

When L2 is presented to the immune system in the context of an L1/L2 co-assembled particle, the L1 response dominates and the L2-specific humoral response is weak or undetectable, consistent with the notion that L2 is poorly accessible on the capsid surface [33,46]. Indeed, humoral responses to L2 are weak and uncommon in HPV infected individuals, whereas L1-specific responses are frequently detected [47]. Here we show that when the two capsid antigens are simply mixed they behave essentially independently as measured by L1 VLP and multimeric L2 ELISA.

One possible approach to broaden protection is the addition of a multimeric L2 polypeptide to the currently licensed HPV vaccines, but it is critical that such mixing does not negatively impact protection against HPV types used to generate the L1 VLP in the vaccine. The ELISA data suggests that mixing L2 11–88 × 5 with Cervarix would not negatively impact the HPV16 L1 VLP-specific antibody response and the HPV16 and HPV18 neutralizing antibody titers did not change, suggesting that L1-mediated protection would remain as effective and that L2-mediated HPV16 neutralization titers were negligible in comparison to the L1-specific response. The L2-specific ELISA titer in sera of mice vaccinated with Cervarix mixed with L2 11–88 × 5 was robust and indistinguishable from that of mice vaccinated with L2 11–88 × 5 alone, suggesting that the L1 VLP did not further adjuvant the L2 response in the presence of alum + MPL. However, the neutralizing antibody titers for two HPV types not used to make the vaccine antigen were weaker than those obtained by vaccination with L2 11–88 × 5, suggesting that there is some dominance of the L1 VLP-specific response over L2 when mixing multimeric L2 with L1 VLP.

The current cost of the licensed HPV vaccines is a major issue preventing implementation in the low income countries where they are most needed. L1 capsomeres produced in E. coli have great potential as an inexpensive alternative to the licensed HPV vaccines. Although L1 capsomeres like L1 VLP, generate a high titer neutralizing antibody response and protect animals from experimental challenge with the homologous virus, there is some suggestion that they are less immunogenic than L1 VLP. Here we compared the serum titers of HPV16 L1 VLP-reactive antibodies and HPV16 neutralizing antibody induced by vaccination with 2 μg HPV16 L1 as VLP versus 2 μg GST-HPV16 L1 capsomeres, each in alum + MPL. We observed 10-fold higher titers with the L1 VLP than the capsomere preparation. Nevertheless, it is possible that similar neutralization titers could be achieved using higher doses of L1 capsomeres, different L1 deletions, L1 capsomeres without the GST tag or a different adjuvant or an additional dose, and further studies are planned to address this issue. We note that the source of both the alum and MPL was different in each preparation, and that Cervarix, which was the source of the HPV16 L1 VLP, also contained a similar quantity of HPV18 L1 VLP, suggesting that a more direct comparison should be made. Indeed, in such an experiment Schadlich et al. observed equivalent immunogenicity between the HPV16 L1Δ10 capsomeres and L1 VLP [25]. Further-
more, even though the titers induced by L1 caposomes were lower in this study, it is likely far above that required to provide effective protection [21]. Here we show that mixing high doses of L2 11–88 × 5 with HPV16 L1 caposomes generated HPV16 neutralizing antibody titers above that of caposomes alone. Although they did not reach the HPV neutralizing antibody titer achieved with Cervarix at 2 weeks post-immunization, at 4 months the gap was closed.

Like VLP L1 caposomes induced a type-restricted response, as no neutralizing antibodies against HPV18, HPV35, HPV45 or HPV58 were detected in the sera of mice vaccinated with HPV16 L1 caposomes. It is surprising that no HPV45 neutralizing antibodies were detected in mice vaccinated with Cervarix, which containing L1 VLP derived from HPV18, the type most closely related to HPV45. Notably low levels of HPV45 neutralizing antibodies have been detected in rabbits and humans vaccinated with HPV18 L1 VLP, and patients vaccinated with Cervarix show significant protection against HPV45 [15–17]. This may reflect a species difference in the nature of the antibody response to HPV18 L1 VLP, that extremely low titers are sufficient for protection, and/or that the neutralization assay lacks sensitivity.

In addition to boosting the HPV16 neutralizing antibody titer, mixing of L2 11–88 × 5 with L1 caposomes also induced HPV45 and HPV58 neutralizing antibodies, similar in titer to sera of mice immunized with L2 11–88 × 5 alone at 0.5 and 4 months. This suggests that the L1 caposomes, unlike the L1 VLP, may not interfere with L2-specific response, and that mixing HPV16 L1 caposomes with a multimeric L2 polypeptide may be an inexpensive strategy to broaden the immunity to diverse HPV types and strengthen the protection against HPV16. However, L2-based prophylactic vaccines have not been tested in patients, and if protective, it is unclear how long such immunity would last.

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