CONCISE COMMUNICATION

Neutralization of Human Papillomavirus Type 11 (HPV-11) by Serum from Women Vaccinated with Yeast-Derived HPV-11 L1 Virus-like Particles: Correlation with Competitive Radioimmunoassay Titer


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Neutralization of human papillomavirus type 11 (HPV-11) has been demonstrated using serum and cervical secretions from primates vaccinated with virus-like particles (VLPs). Theoretically, neutralizing antibodies could protect women from HPV infection. The immunogenicity of a yeast-derived HPV-11 L1 VLP vaccine was tested in women. Serum specimens were evaluated for HPV-11 titer by competitive radioimmunoassay (cRIA) and for neutralization by use of the athymic mouse xenograft system. Analysis of serum from 104 subjects showed a dose response in HPV-11 cRIA titers and neutralization. Overall, 68 (82.9%) of 82 postimmunization serum specimens from VLP recipients were 100% neutralizing when used in the assay at a 1:50 dilution. Of 69 serum specimens, 63 (91.3%) with cRIA titers >200 milliMerck units per milliliter were neutralizing. Immunization with HPV VLPs elicits a vigorous serum immune response in a high percentage of women. The HPV-11 cRIA titer appears to be a surrogate marker for neutralization.

Human papillomaviruses (HPVs) infect genital, respiratory, and cutaneous epithelia, causing a range of disease states, including genital warts and malignant lesions of the uterine cervix. HPV types 6 and 11 are present in ~95% of genital warts; HPV types 16, 18, and 31 and other “high-risk” types are present in ~95% of cervical carcinomas [1, 2]. Treatment for most clinically apparent lesions is limited to physical or chemical destruction, which are presumably ineffective treatments, as evidenced by the recurrence rate resulting from insufficient eradication of HPV-infected cells. Therefore, a prophylactic vaccine is needed that could prevent HPV infection.

Vaccine development has been slowed by difficulties in propagating HPV in tissue culture and in infecting nonhuman species. Several HPV types have been propagated in the athymic mouse xenograft system [3]. Expression of the L1 gene in yeast results in the formation of virus-like particles (VLPs) that resemble native virions [4]. Immunization with VLPs elicits virus-neutralizing antibodies that recognize conformational epitopes on native virions [5].

Vaccination of animals with recombinant VLPs has been shown to prevent disease after challenge with the same virus type [6–8]. We showed earlier that serum from primates immunized with HPV-11 VLPs neutralized HPV-11 virions [9]. In addition, blood-free cervical secretions collected from the animals partially neutralized HPV-11 virions [9]. On the basis of these data, the immunogenicity of an HPV-11 VLP (yeast derived) vaccine was evaluated in college-age women. Vaccine was administered in a double-blind, dose-escalating manner. Women who tested negative for HPV types 6 and 11 were vaccinated with HPV-11 VLP vaccine. Serum from each subject was evaluated for antibody titer to HPV-11 and for neutralization of HPV-11 virions.

Materials and Methods

Vaccination and collection of serum specimens. Women were screened for HPV types 6 and 11 (HPV-6/11) infection by polymerase chain reaction (PCR) analysis of swab samples of multiple anogenital sites. In addition, competitive RIA (cRIA) and ELISA [10] were used to measure antibodies to HPV-6/11 in serum specimens. Young women (18–25 years old) who tested negative for HPV-6/11 by PCR and had no detectable antibodies to HPV-6/11 by ELISA and to HPV-11 by cRIA were randomized to receive placebo or HPV-11 L1 VLP vaccine (10, 20, 50, or 100 µg of VLP
protein (Merck). Equal doses of vaccine or placebo were administered by intramuscular injection at enrollment and after 2 and 6 months. Serum specimens were evaluated before and after vaccination for antibodies to HPV-11 by cRIA and for HPV-11 neutralization by using the athymic mouse xenograft system [11].

RIA. The HPV-11 antibody cRIA is a quantitative assay based on competition between HPV-11 antibodies in serum specimens and an HPV-11 monoclonal antibody (MAb 8740) [12]. Antibodies compete for binding to limiting amounts of HPV-11 L1 VLP antigen coated onto a solid phase. MAb 8740 has been shown to neutralize HPV-11 [12]. Polystyrene beads were coated with yeast-derived HPV-11 VLPs [13]. Equal volumes of undiluted or diluted immune serum HPV-11 [12].

Petite for binding to limiting amounts of HPV-11 L1 VLP antigen coated onto a solid phase. MAb 8740 has been shown to neutralize HPV-11 [12]. Polystyrene beads were coated with yeast-derived HPV-11 VLPs [13]. Equal volumes of undiluted or diluted immune serum (100 μL) and diluted MAb 8740 (1:160,000 in PBS, 1% bovine serum albumin, and 0.05% Tween 20) were mixed in a 24-well plate (Ab-bott). One HPV-11-coated bead was added to each well, and the assay plates were sealed and were incubated overnight. Beads were washed and were incubated with 125I-labeled goat anti-mouse IgG. Beads were again washed, transferred to carrier tubes, and counted in a gamma counter (Wallac). Relative inhibition of MAb 8740 binding by serum antibody was compared with a standard curve, using a 4-parameter logistic curve fit. The immune reference serum used for the standard curve was assigned an arbitrary value expressed in arbitrary units: milliMerck units (mMU) per milliliter. The cRIA cutoff value was determined relative to the standard curve, using multiple assays of preimmune African green monkey (AGM) serum from an AGM vaccinated with HPV-11 VLPs and serum from uninfected women with a low risk of virus exposure (18–25-year-old women with no history of abnormal results of cervical cytological analysis or genital warts). The cutoff value for the HPV-11 cRIA was established at 10 mMU/mL.

HPV-11 neutralization assay. Serum from an immunized AGM was used as a positive neutralization control, and preimmune serum from this animal was used as a nonneutralizing infectivity control at dilutions of 1:50. The operators were blinded to the subject, immunization schedule, and dose group of each serum specimen. A single foreskin tissue was obtained from a neonatal circumcision and was used for each assay. Foreskin fragments for implantation into 3 mice were combined with 88 μL of MEM (Sigma), 10 μL of HPV-11 virus (≈10^6 virions) [11], and 2 μL of serum from a subject (final dilution, 1:50) and were incubated at 37°C for 90 min. Foreskin fragments then were implanted under the renal capsules of athymic mice. Mice were killed 10 weeks later, and implants were recovered and were fixed in zinc-formalin.

Paraffin-embedded implant sections were analyzed for HPV infection by histological analysis and DNA in situ hybridization. The histological changes used to indicate HPV-11 infection were acanthosis, koilocytosis, and parakeratosis. All 3 changes were required for indication of infection. A typing assay (PathoGene human papillomavirus in situ typing assay; ENZO) was used to localize HPV sequences in implants. Sections from HPV-11–infected implants and a neonatal foreskin tissue were included in each assay as positive and negative controls, respectively.

Criteria for validity and HPV-11 neutralization. Pre- and postimmunization serum specimens from 5 subjects and 2 AGM controls were tested in each assay, using a total of 19 mice. Three mice were used to test each serum specimen, potentially yielding 6 implants. Two mice each were used for the pre- and postimmunization AGM control serum specimens.

Each assay was considered valid if 100% neutralization was achieved using postimmunization control AGM serum and if infection of >50% of growth-control implants was achieved by using preimmunization serum. If any implant derived from the postimmunization control or from <50% of the preimmunization growth-control implants were HPV-11 positive, the assay was declared invalid and was repeated. Overall, 1 assay was repeated because a single postimmunization control implant was HPV-11 positive. Two assays were repeated because <50% of the preimmunization growth-control implants were HPV-11 positive.

DNA in situ hybridization was a more sensitive indicator of infection than was histology and therefore was used in the computation of partial neutralization for each serum sample that did not demonstrate 100% neutralization. Partial neutralization was computed as 1 – (no. of HPV-11–infected implants/total no. of implants), in which the number of infected implants was determined by DNA in situ assay.

After completion of a valid assay, each implant was given a final grade of positive or negative for infection, as determined on the basis of the accumulated data. If both histological and DNA in situ assay results were negative, the implant was recorded as negative for infection. If either histology or in situ assay results were positive, the implant was recorded as positive for infection but not for neutralization. For the DNA in situ assay, a single positive epithelial cell in a section indicated that the implant was HPV-11 infected. Serum samples were retested only if the entire assay was invalid, in which case, all serum specimens in the assay were retested.

Statistical methods. An analysis was conducted on the HPV-11 cRIA and neutralization results for 104 subjects. Subjects who dropped out of the study before month 7 or who were positive for HPV-6 or -11 by PCR at enrollment or at any time through month 7 were excluded from the analysis. The percentages of subjects in each dose group with HPV-11 cRIA titers ≥200 mMU/mL were computed, and 95% confidence intervals (CIs) were constructed. In addition, the geometric mean titers (GMTs) for each dose group were computed for each group with 95% CIs. The percentages of subjects with neutralizing (total or partial) serum specimens were computed, and graphics were created to illustrate the association between HPV-11 cRIA titers and neutralization.

Results

Dose response in HPV-11 titers. Serum from 104 subjects was evaluated. Details of the safety and tolerability of the vaccine and of the persistence of antibody response will be reported at a later time. No subject in the placebo group developed a measurable HPV-11 antibody titer, as determined by cRIA. A dose response was observed in cRIA titer, with an apparent doubling of the month 7 GMTs between the 10- and 20-μg groups (table 1). A further doubling was observed between the 50- and 100-μg groups (table 1). Month 7 GMTs of 258, 644, 647, and 1112 mMU/mL were recorded for the 10-, 20-, 50-, and 100-μg groups, respectively. The lower bound of the 95% CI of month 7 cRIA
titers for the 20-, 50-, and 100-μg groups exceeded 200 mMU/mL, a titer that correlated with neutralization (see below).

**Dose response for HPV-11 neutralization.** Pre- and postimmunization (month 7) serum from each of 104 subjects was tested for neutralization. The criterion for neutralization, as described above, was strict. Table 1 indicates that a dose response occurred when the criterion of 100% neutralization was used. Also included in the table are the percentages of serum specimens found to be partially neutralizing.

No preimmunization serum from any patient was neutralizing. One (4.5%) of 22 postimmunization serum specimens from placebo-vaccinated patients was neutralizing. This subject did not develop detectable antibodies to HPV-11 VLPs by cRIA. The remaining 21 placebo-only recipients did not develop neutralizing antibodies. For vaccine recipients, 68 (82.9%) of 82 postimmunization serum specimens were 100% neutralizing, and dose responses of 68.4%, 78.9%, 87.5%, and 95.0% were observed for the 10-, 20-, 50-, and 100-μg groups, respectively (table 1).

**Correlation of neutralization with HPV-11 cRIA titer.** The HPV-11 cRIA titer correlated well with neutralization (figure 1). Only 1 neutralizing serum specimen had a cRIA titer of <100 mMU/mL. The GMT of all neutralizing serum specimens was 718 mMU/mL. A cRIA titer of >200 mMU/mL correlated with neutralization (figure 1). Sixty-three (91.3%) of 69 serum specimens with cRIA titers >200 mMU/mL were neutralizing (not significantly different from 82.9% [68/82], the overall percentage of postimmunization serum specimens that were 100% neutralizing). In addition, a dose response was observed for the percentage of immunized recipients with cRIA titers >200 mMU/mL: 52.6%, 89.5%, 91.7%, and 100% for the 10-, 20-, 50-, and 100-μg groups, respectively (figure 1).

**Partial neutralization.** Thirteen serum specimens partially neutralized (defined as <100% but ≥50% neutralization) HPV-11 virions (table 1). HPV-11 cRIA titers of the 11 VLP-vaccinated subjects ranged from 50 to 1520 mMU/mL: titers for 4, 6, and 1 were <100, 100–1000, and >1000 mMU/mL, respectively. The remaining 2 partially neutralizing serum specimens were from placebo-immunized subjects, and these subjects did not develop a cRIA antibody response.

**Table 1.** Month 7 human papillomavirus type 11 RIA geometric mean titers, in milliMerck units per milliliter, with 95% confidence intervals, and percentages of neutralizing serum specimens from groups of patients vaccinated with various doses of virus-like particles (VLPs).

<table>
<thead>
<tr>
<th>Group, dose (μg)</th>
<th>Geometric mean titer (95% confidence interval)</th>
<th>Neutralizationa</th>
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<tbody>
<tr>
<td></td>
<td>100%</td>
<td>≥75%</td>
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<tr>
<td>Placebo (22)</td>
<td>5.0 (5.0–5.0)</td>
<td>1 (4.5)</td>
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<tr>
<td>VLP vaccinated</td>
<td></td>
<td></td>
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<tr>
<td>10 μg (19)</td>
<td>258.4 (162.9–409.9)</td>
<td>13 (68.4)</td>
</tr>
<tr>
<td>20 μg (19)</td>
<td>644.1 (406.3–1021.2)</td>
<td>15 (78.9)</td>
</tr>
<tr>
<td>50 μg (24)</td>
<td>647.4 (448.9–933.7)</td>
<td>21 (67.5)</td>
</tr>
<tr>
<td>100 μg (20)</td>
<td>1118.0 (793.5–1557.6)</td>
<td>19 (95.0)</td>
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* Data are no. (%) of patients.

**Discussion**

In this study, a high percentage of women vaccinated with HPV-11 VLPs developed serum antibodies capable of neutralizing large numbers of HPV-11 virions. The neutralizing titers were high enough that serum specimens could be diluted 1:50 and still completely neutralize purified virus. The cRIA titer against HPV-11 correlated well with neutralization of large quantities of virus. Two other recent reports also indicate that HPV VLPs are effective in inducing serum antibody responses in humans [14, 15].

In summary, this analysis demonstrates that vaccination with HPV-11 VLPs elicits a vigorous serum immune response in a high percentage of college-age women. A dose response was observed for HPV-11 cRIA titers and for HPV-11 neutralization. Serum samples with HPV-11 cRIA titers >200 mMU/mL were neutralizing in most cases. This level of antibody occurred most often in women vaccinated with 20, 50, or 100 μg of HPV-11 VLPs. The HPV-11 cRIA titer correlated well with HPV-11 neutralization and could potentially be used as a surrogate marker for neutralization of HPV-11 in larger vaccine trials. Future studies will evaluate the efficacy of VLP vaccines in protecting against genital HPV infection.

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**References**


