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Assessment of HPV 16 and HPV 18 antibody responses by pseudovirus neutralization, Merck cLIA and Merck total IgG LIA immunoassays in a reduced dosage quadrivalent HPV vaccine trial☆

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ABSTRACT

We assessed HPV 16 and 18 antibody responses of female subjects enrolled in a 2- vs. 3-dose quadrivalent HPV (Q-HPV) vaccine trial (ClinicalTrials.gov NCT00501137) using the Merck competitive Luminex (cLIA) and total IgG Luminex (TIgG) immunoassays, and a pseudovirus neutralizing antibody (PsV NAb) assay. Subjects were enrolled in one of three groups: (1) 9–13 yr, 2 doses of Q-HPV at 0, 6 months ($n = 259$); (2) 9–13 yr, 3 doses at 0, 2, 6 months ($n = 260$); and (3) 16–26 yr, 3 doses at 0, 2, 6 months ($n = 305$). Sera were collected from all subjects at baseline, months 7 and 24, and from half the subjects at months 18 and 36. High correlation was observed between all three assays. At month 36, HPV 16 antibodies remained detectable in all subjects by all assays, whereas 86.4%, 99.6% and 100% of subjects respectively were HPV 18 cLIA, TIgG and PsV NAb (partial neutralization endpoint) seropositive. The proportion seropositive for HPV 18 by cLIA at 36 months was not significantly different for 2-dose girls vs. 3-dose adults (85.9% vs. 79.4%; $p = 0.51$), whereas the proportion for 3-dose girls was significantly higher than for 3-dose adults (95.3% vs. 79.4%; $p < 0.01$). The HPV 18 seropositive proportions by the TIgG and PsV NAb (partial neutralization endpoint) assays were the same for all subjects. High baseline HPV 16 and HPV 18 seropositivity was observed for the TIgG assay and it is unclear if all the detected TIgG antibodies are type-specific and/or neutralizing. For the PsV NAb assay, 90% and partial neutralization geometric mean titres were consistently 2–8-fold higher than for 100% neutralization, which enabled detection of HPV 18 NAb in subjects who lost detectable cLIA antibodies over time. We conclude that the PsV NAb assay is more sensitive than the cLIA, and likely more specific than the TIgG assay.

Abbreviations: cLIA, Merck competitive Luminex immunoassay; EIA, enzyme immunoassay; GMT, geometric mean titre; mMU, milli-Merck units; NT_{100}, 100% neutralization endpoint; NT_{90}, 90% neutralization endpoint; $NT_{partial}$, partial neutralization endpoint; PsV NAb, pseudovirus neutralizing antibody; Q-HPV, quadrivalent HPV; RFP, red fluorescent protein; TIgG, Merck total IgG Luminex immunoassay; VLP, virus-like particle.

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1. Introduction

Human papillomavirus (HPV) vaccines induce type-specific neutralizing antibodies which correlate with immunity to the corresponding HPV types [1], and World Health Organization guidelines recommend that assays which assess neutralization be used as the reference standard for measuring HPV vaccine responses [2]. Quadrivalent HPV (Q-HPV) vaccine (Gardasil®, Merck Laboratories) consists of HPV 6, 11, 16 and 18 virus-like particles (VLP) and is licensed for a 3-dose regimen. Post-Gardasil® antibody responses are typically measured by a proprietary multiplex competitive Luminex immunoassay (cLIA) [3], which is based on competitive binding of type-specific HPV antibodies in human sera with labelled monoclonal antibodies directed against neutralizing epitopes of the respective VLP types (HPV 6, 11, 16 and 18). It has been reported that HPV antibodies measured by the cLIA may decline to become undetectable over time, especially for HPV 18, despite continued vaccine efficacy in preventing infections [4,5]. The significance of the loss of detectable antibodies is unknown as protective levels of HPV antibodies remain undefined [1,6,7] and vaccine efficacy remains near 100%. Recently, Merck Laboratories developed a total IgG Luminex immunoassay (TIgG) which measures antibodies against the entire VLP, i.e., a broader array of VLP antibodies than the cLIA, for nine HPV types (HPV 6, 11, 16, 18, 31, 33, 45, 52 and 58) [8].

In order to further characterize HPV antibody responses in a 2- vs. 3-dose randomized controlled Q-HPV vaccine trial, we adapted and implemented the National Institutes of Health pseudovirus neutralizing antibody (PsV NAb) assay [9], in which a red fluorescent protein (RFP) reporter plasmid was incorporated into the PsV [10]. Neutralizing antibodies block PsV entry into susceptible cells and prevent expression of the RFP which is visualized by fluorescence microscopy. While PsV NAb assays are technically complex and have not been standardized, they provide an alternative to vaccine manufacturers' assays by detecting type-specific antibodies that block HPV infection of susceptible cells.

We previously reported HPV 16 and 18 PsV NAb and cLIA responses for the 2- vs. 3-dose trial at 7 months post-vaccination [11]. We now report HPV 16 and HPV 18 PsV NAb, Merck cLIA and Merck TIgG antibody responses through to 36 months post-vaccine.

2. Materials and methods

2.1. Study population

The study population consisted of 824 females aged 9–26 years at three study sites in Canada (British Columbia, Québec and Nova Scotia), who were enrolled into one of three study arms as previously described [12]. Younger subjects (9–13 yr) were randomly assigned to receive two or three doses of Q-HPV vaccine, whereas older subjects (16–26 yr) received only the standard three dose regimen. Distribution among the study arms was: Group 1 (n = 259), 9–13 yr (mean age 12.4 yr), received two doses at months 0 and 6; Group 2 (n = 260), 9–13 yr (mean age 12.3 yr), received three doses at months 0, 2 and 6; and Group 3 (n = 305), 16–26 yr (mean age 19.3 yr), received three doses at months 0, 2 and 6 (Fig. 1). Sera were collected from the entire cohort at baseline, months 7 and 24; in addition, half the cohort was randomly selected for serum collection at month 18, and the other half had serum collected at month 36. Group 3 subjects also provided self-collected vaginal swabs (HC^{TM} Female Swab Specimen Collection Kit; Qiagen) to determine if HPV 16 or HPV 18 DNA positivity at baseline impacted the respective antibody responses.

Informed consent was obtained for all subjects after explaining the nature and possible consequences of the study. The study was approved by the University of British Columbia Clinical Research Ethics Board and by local research ethics boards at the other sites. The clinical trial was registered with ClinicalTrials.gov (NCT00501137).

2.2. HPV antibody assays

The PsV NAb assay was performed as previously described [10]. Briefly, HPV 16 and 18 PsV incorporating RFP were prepared by transfection of 293TT cells with HPV 16 or 18 L1 and L2 plasmids together with RFP plasmids. PsV preparations were purified and titrated in 293TT cells. The PsV L1 protein concentrations were estimated by comparing polyacrylamide gel electrophoresis L1 band densities for each PsV preparation with the densities of known concentrations of HPV 16 and 18 Merck vaccine VLPs. The HPV 16 PsV contained approximately 2–3-fold more L1 compared to the HPV 18 PsV.

Subject sera were serially diluted, mixed with 100 infectious units of the respective HPV 16 or 18 PsV, and inoculated onto 293TT

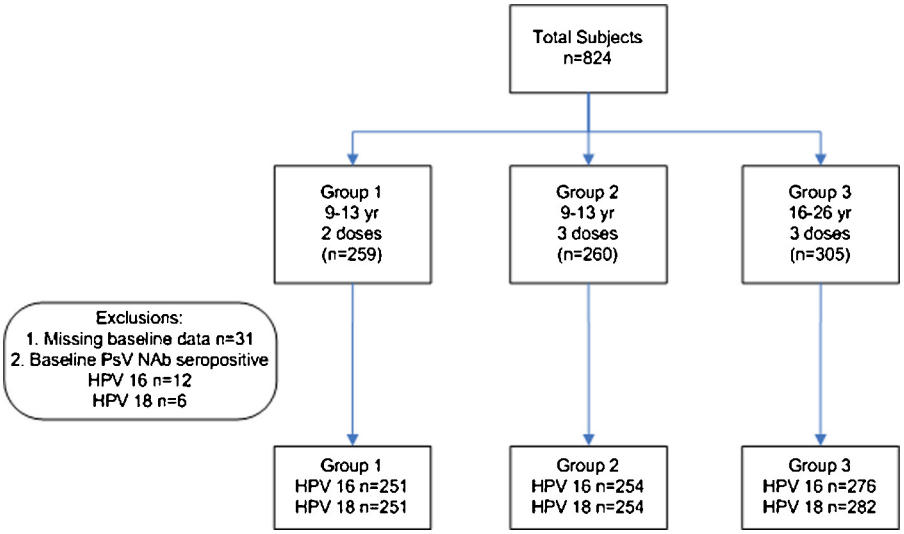

Fig. 1. Distribution of study subjects. Distribution of study subjects by age group and dosing regimen, and description of exclusions. *Abbreviation*: PsV NAb, pseudovirus neutralizing antibody.