Vaccine 32 (2014) 3540–3547

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Low doses of flagellin-L2 multimer vaccines protect against challenge with diverse papillomavirus genotypes

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ARTICLE INFO

Article history:
Received 12 November 2013
Received in revised form 16 March 2014
Accepted 14 April 2014
Available online 26 April 2014

Keywords:
Human papillomavirus
Vaginal challenge
Cutaneous challenge
L2
HPV
Prophylactic vaccine
Neutralizing antibody
Protective efficacy

ABSTRACT

Genetically modified bacterial flagellin (Fla), a Toll-like receptor-5 (TLR5) ligand, was evaluated as a fusion partner for human papillomavirus (HPV) L2-based immunogens in two animal challenge models; either cutaneous inoculation of rabbits with HPV 'quasivirions' containing cottontail rabbit papillomavirus (CRPV) genomes that induce warts, or intra-vaginal inoculation of mice with HPV 'pseudovirions' encapsidating a luciferase reporter plasmid and measurement of bioluminescence to determine infectivity. An *Escherichia coli* production system was developed for flagellin-L2 (Fla-L2) fusions containing either monomeric HPV-16 L2 a.a. 11(× 11–200) or oligomeric L2 comprising a fusion of the a.a. 11–88 peptides of five (Fla~5 × 11–88) or eight (Fla~8 × 11–88) genital HPV types. Immunogenicity and bioactivity of Fla-L2 constructs were assessed using an in vitro neutralization and cell-based TLR-5 binding assay, respectively. Efficacy was evaluated following active immunization of rabbits or mice administered 3 intramuscular doses of Fla-L2 recombinants without exogenous adjuvant, followed by challenge. In addition, passive immunization studies of naïve rabbits with serial dilutions of pooled immune sera were used to determine End-Point Protection Titers (EPPT) for each formulation against a broader spectrum of HPV quasivirions. Efficacy was assessed for up to 10 weeks on the basis of wart volume induced following challenge and results compared to licensed L1-VLP vaccines (Gardasil and Cervarix). Following active immunization at doses as low as 1 μg, Fla-L2 fusions afforded complete protection against infection (mice) and disease (rabbits) following either homologous or heterologous HPV challenge. Passive immunization with anti-L2 immune sera discriminated between the different vaccine candidates under evaluation, demonstrated the protective role of antibody and suggested the superiority of this oligomeric L2-TLR5 agonist fusion approach compared to L1-based vaccines in its ability to cross-protect against non-vaccine HPV types.

1. Introduction

Over 120 identified HPV types have been classified into five genera: Alpha-, Beta-, Gamma-, Mu- and Nu-papillomaviruses [1]. Most alpha HPV types infect the genital tract and are sexually transmitted. Within the alpha papillomaviruses, fifteen genotypes [2–8] are classified as "high-risk" (HR-HPV) and are considered the causal agents of cervical cancer [9] (Fig. 1A). Cervical cancer represents 9% of cases of female cancer and is the third leading cause of cancer in women worldwide, with more than 529,000 new cases and 275,000 deaths per year [10], and 99% of cases contain HPV DNA [11,12].

Both HPV vaccines, Gardasil (Merck & Co. Inc.) and Cervarix (GlaxoSmithKline), are based upon virus-like particles (VLPs) derived from major capsid protein L1 and are licensed for protection against the two HPV types (HPV-16 and HPV-18) that cause 70% of cervical cancers, 80% of anal cancers, 60% of vaginal cancers, and 40% of vulvar cancers. Gardasil also targets the two HPV types that cause 90% of benign genital warts, HPV-6 and HPV-11. Although there is accumulating evidence suggesting that HPV vaccination also confers some cross-protection against types

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http://dx.doi.org/10.1016/j.vaccine.2014.04.032
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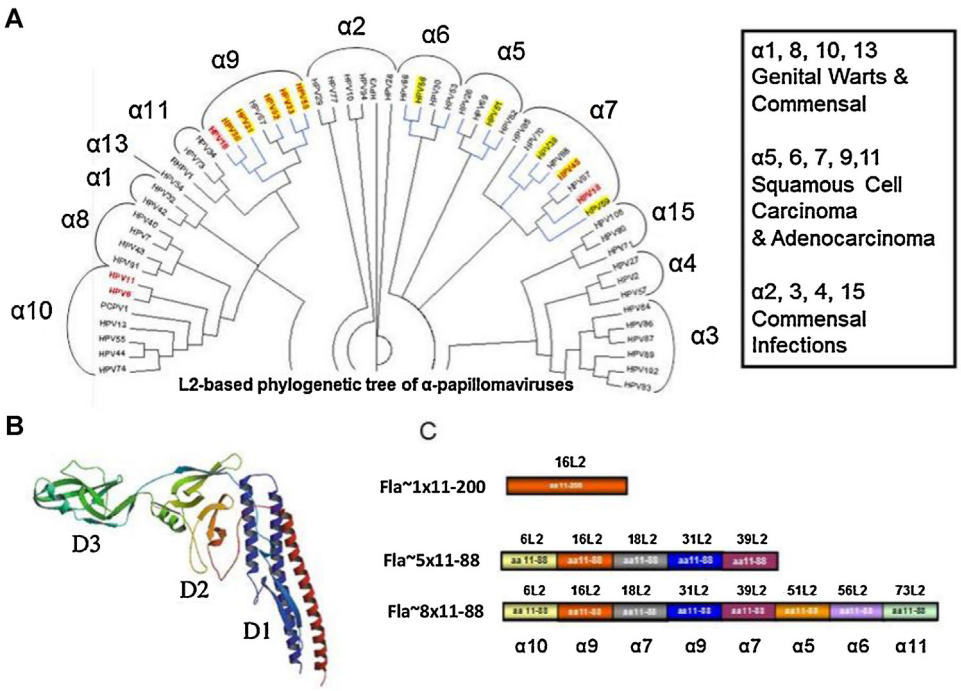

Fig. 1. Phylogenetic tree of alphapapillomavirus and design of Fla-L2 vaccines. (A) Phylogenetic tree of alpha papillomavirus based on L2 amino acid sequences was built using the neighbor-joining Jukes-Cantor method [1]. The final tree is a consensus made from bootstrap resampling of 100 replicates. The circular representation was generated using Dendroscope [2]. Bold red font indicates HPV types represented in nona (9)-valent Merck vaccine (NCT00543543); blue lines indicate HPV types associated with cancers; pink background indicate HPV types against which licensed vaccines are ~100% protective; yellow background indicates HPV types reported to be weakly cross-protected by licensed vaccines. Alpha clade numbering is designated over the brackets. (A) (inset) – General characteristics of alpha clades (cited from Schiffman et al. [3]). (B) 3D models of Flagellin modified from [4]. Domain D1, D2, D3 domains are depicted by color. (C) A diagram showing the composition of L2 inserts for individual Fla-L2 vaccine candidates. Individual subunits were derived from different HPV species. Specific α-clades for each type represented in vaccine is depicted below Fla~8 × 11–88 L2 diagram. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

most closely related to those used to produce the VLPs, efficacy against non-vaccine non-alpha-7/9 HPV types is significantly lower [13,14], and this cross-protection may potentially wane faster than immunity to the vaccinal types HPV16 and HPV18 [15]. In addition, the non-vaccine HR-HPV types tend to induce cervical intraepithelial neoplasia grade 3+ (CIN3+) more slowly than the vaccine-targeted types(9), which implies that CIN3+ caused by slow progressor HPV types may account for a higher percentage of CIN3+ over time [16]. Therefore a new generation of broader spectrum HPV vaccines is being developed, including a 9-valent L1-VLP vaccine including 7 HR-HPV types 16, 18, 31, 33, 45, 52 and 58, and the low risk types HPV6 and 11 (Merck & Co. Inc., NCT00543543). A potential benefit to the development of the 9-valent vaccine is to lower the continuing need to screen after vaccination.

The potential impact of widespread HPV vaccination in developing countries, wherein 80% of cervical cancer cases occur, is enormous, but the current cost of HPV vaccination remains a barrier to their introduction [17]. Reduced dosing schedules for the L1-based vaccines may be sufficient and reduce cost [18]. HPV vaccines are licensed in many low- and middle-income countries, although few have established national immunization programs. Although tiered pricing and 2-dose regimens have potential to greatly expand access, an affordable HPV vaccine covering the majority of HR-HPV incident infections is still urgently needed [19].

A cost-effective HPV vaccine based on a single antigen *Escherichia coli*- produced minor capsid protein L2 might address this problem. Vaccination with the N-terminus of the L2 protein protects animals from experimental challenge with either animal papillomaviruses [19–21] or HPV pseudovirions that carry a reporter plasmid [2,20]. The N-terminus of L2 does not assemble into a VLP but does effectively present its linear protective epitopes when fused in tandem with the same region of several HPV types [22]. Indeed immunization with such concatemers/multimers of L2 derived from several high risk HPV types, induces neutralizing antibodies that protect mice from vaginal HPV challenge by diverse genotypes [22] despite eliciting neutralization titers significantly lower than L1 VLP vaccines [23].

Engagement of TLRs by their cognate agonists and the subsequent signaling within antigen presenting cells (APC) leads to enhanced processing and presentation of antigens that are co-delivered to those APC [24,25,26]. A TLR-2 agonist was required to adjuvant a short L2 epitope (HPV16; AA 17–36) linked to a universal T-helper epitope and provided mice protection against heterologous HPV challenge [2]. Further, use of an adjuvant with L2 multimer vaccination is an important factor in obtaining effective protection [22], and inclusion of a TLR agonist, such as monophosphoryl lipid A (MPL) or CpG, with 1 μg L2 multimer formulated in alum can provide dose sparing [27].

The principle of utilizing flagellin as a carrier/adjuvant is well described [28–31]. The adjuvant property of flagellin is mediated by TLR5, linking innate and adaptive immunity via MYD88 and TRAF6, leading to NF-κB activation, cytokine secretion and an inflammatory response [28,29,32,33]. Epitope based vaccines delivered via fusion with flagellin are efficacious against a number of viral [34–36] and bacterial [37,38] targets. The safety and ability to induce protective levels of serum antibody have been demonstrated in preclinical [4,5,34–36,39–41] as well as in recent clinical studies [42,43] of flagellin-based candidate influenza vaccines. Therefore fusion with flagellin, which offers a combination of TLR activity and T-helper epitopes, was examined as a self-adjuvanting carrier for L2.