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Immunogenicity of next-generation HPV vaccines in non-human primates: Measles-vectored HPV vaccine versus *Pichia pastoris* recombinant protein vaccine

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ABSTRACT

Human papillomavirus (HPV) infection is the most common sexually transmitted disease worldwide. HPVs are oncogenic small double-stranded DNA viruses that are the primary causal agent of cervical cancer and other types of cancers, including in the anus, oropharynx, vagina, vulva, and penis. Prophylactic vaccination against HPV is an attractive strategy for preventing cervical cancer and some other types of cancers. However, there are few safe and effective vaccines against HPV infections. Current first-generation commercial HPV vaccines are expensive to produce and deliver.

The goal of this study was to develop an alternate potent HPV recombinant L1-based vaccines by producing HPV virus-like particles into a vaccine that is currently used worldwide. Live attenuated measles virus (MV) vaccines have a well-established safety and efficacy record, and recombinant MV (rMV) produced by *reverse genetics* may be useful for generating candidate HPV vaccines to meet the needs of the developing world.

We studied in non-human primate rMV-vectored HPV vaccine in parallel with a classical alum adjuvant recombinant HPV16L1 and 18L1 protein vaccine produced in *Pichia pastoris.* A combined prime-boost approach using both vaccines was evaluated, as well as immune interference due to pre-existing immunity against the MV.

The humoral immune response induced by the MV, *Pichia*-expressed vaccine, and their combination as priming and boosting approaches was found to elicit HPV16L1 and 18L1 specific total IgG and neutralizing antibody titres. Pre-existing antibodies against measles did not prevent the immune response against HPV16L1 and 18L1.

1. Introduction

Persistent infections sustained by "high-risk" genotypes of human papilloma viruses (HPVs) lead to the development of high-grade squamous intraepithelial lesions and ultimately cause cervical cancer (CC). Worldwide, approximately 70% of CCs are caused by the HPV16 or HPV18 serotypes; the remaining 30% are caused by at least 10 other high-risk HPV subtypes [1–3].

L1, the major capsid protein of HPV, capable to self-assemble into virus-like particles (VLPs), is a type-specific and highly immunogenic antigen, and can elicit high titres of neutralizing antibodies, conferring protection against HPV infection [4,5].

Currently, prophylactic HPV vaccines based on L1 structural proteins are well established to prevent CC. The two licensed HPV vaccines, quadrivalent Gardasil from Merck (qHPV-6/11/16/18 vaccine; Kenilworth, NJ, USA) and bivalent Cervarix form GlaxoSmithKline (bHPV-16/18 vaccine; Brentford, UK), were approved in many countries and found to be safe and effective for preventing infection by vaccine-related HPV serotypes [6]. However, administration has been virtually non-existent in developing countries, mainly because of the prohibitively high cost

Abbreviations: HPV, human papilloma virus; CC, cervical cancer; MV, measles virus; rMV, recombinant MV; EZ, Edmonston Zagreb strain; IFN-ϒ, interferon gamma; IL-5, interleukin 5; IC_{50}, concentration that gives a response halfway between baseline and maximal; N, nucleocapsid protein; P, phosphoprotein; M, matrix protein; F, fusion protein; H, attachment protein; NNSV, nonsegmented negative strand RNA virus.

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of the vaccine and difficulties in implementing vaccination programs, which require three injections for adolescent girls, over a six-month period [6]. The recently approved nonavalent vaccine (Gardasil 9, Merck) provides protection against 5 additional oncogenic types (HPV 31, 33, 45, 52, 58) [7,8]; however, it possesses the same difficulties as its predecessor.

Therefore, next-generation HPV vaccines are required to reduce production costs and improve immunization schedule feasibility.

This study aimed to develop a cost-effective, safe, and effective prophylactic HPV vaccine to address the needs of developing countries. We developed a recombinant HPV vaccine based on a live attenuated measles virus (MV)-vectored platform [9]. Additionally, we compared its immune response to that of the classical *Pichia pastoris*-produced HPV16L1 and 18L1 recombinant vaccines in non-human primates.

Live attenuated MV vaccines have a well-established safety and efficacy record and are inexpensively produced and delivered to most developing countries. MV vaccines induce life-long immunity after a single injection, and reversion to pathogenicity has never been observed [10]. In 2014, the WHO estimated that the global immunization coverage with at least the first dose of live attenuated MV vaccine was higher than 85%.

MV is an enveloped virus with a nonsegmented, negative-sense single-stranded RNA genome of 15,894 nucleotides in length. The genome contains six transcription units, which are separated by nontranscribed trinucleotide intergenic sequences and are flanked by 3′ leader and 5′ trailer sequences at the genome ends. The MeV genome encodes a total of eight proteins. The six structural proteins are the nucleocapsid protein (N), phosphoprotein (P), matrix protein (M), fusion protein (F), attachment protein (H), and the large RNA-dependent RNA polymerase protein (L). Two additional nonstructural proteins (C and V) are encoded in the P transcription unit [11].

Recombinant-MVs (rMV) produced by reverse genetics [12,13] represent an attractive platform for generating multivalent vaccines to immunize against measles and other infectious agents or to carry therapeutic agents [14–16]. As a proof of concept, MVs have been used to express antigens derived from HIV [17,18], hepatitis B [14], West Nile virus [19], SARS coronavirus [20], and Chikungunya virus [21,22]. In most of these studies, rMVs expressing heterologous antigens appeared to induce long-term specific neutralizing antibodies, even in the presence of pre-existing immunity against MV [23,24]. In a previous study, our rMV-HPV16L1 vaccine candidate was found to effectively induce specific neutralizing antibodies against HPV16L1 [9,25] in transgenic CD46IFNAR mice susceptible to MV infection [26]. Here, we examined the development of the recombinant live attenuated MV Edmonston-Zagreb (rMVEZ) strain as a viral vector to carry heterologous genes encoding the major capsid protein L1 of HPV-type-16 and HPV-type-18 in rhesus monkeys and compared their immunogenicity in a traditional methylotrophic *P. pastoris*-based expression system [27].

2. Materials and methods

2.1. Cloning, rescue, characterization and production of measles vectored HPV L1 candidate vaccine viruses

Human-codon optimized genes coding for the HPV16L1 and HPV18L1 antigens were chemically synthesized at GeneArt™ (Thermo Fisher Scientific, USA). Plasmid p(+)MVEZ, delivering correctly T7-initiated and ribozyme-terminated MV antigenome, was constructed as previously described [12]. *BssH*II-*Aat*II cloning of the synthetic genes as additional transcription units was in position 2 (between the P and M protein genes) of p(+)MVEZ [Fig. 1]. The methods used for cloning were similar to those described by Sambrook and Russell [28]. The ligated products were transformed into *Escherichia coli* XL10-Gold (Stratagene, USA) Ultra Competent cells. Growing colonies selected by ampicillin (100 μg/mL) resistance were screened by *BssH*II-*Aat*II digestion after minipreparation of plasmid DNAs, following the Qiagen Plasmid Purification protocol. Recombinant positive clones, named p(+)MVEZHPV16L1 and p(+)MVEZHPV18L1, were used to transform XL10-Gold cells, amplified by maxipreparation following the Qiagen Plasmid Purification protocol, assayed by analytical digestion, and confirmed by primer-walking sequencing (Eurofins MWG Biotech, Germany). The rMVEZHPV16L1 and rMVEZHPV18L1 viruses were then rescued by 293T cells transfection in a five-plasmid transfection system. Single syncytia were expanded in the MRC-5 cell line to establish passage 0 (P0); the recombinant viruses were serially passaged to prepare sufficient virus stocks. The rMVEZHPVs were quantified by CCID50 microtitration in 96-well plates by Spearman and Karber formula [29].

Media and cell lysate from Vero cells infected with either rMVEZHPV16L1 or MVEZHPV18L1 were lysed at 48 h post-infection. They were analyzed by 10% SDS–PAGE to determine the expression of HPV-specific L1 antigen by western blotting. HPV16L1 and HPV18L1 were detected with specific mouse monoclonal antibodies (Abcam, UK; Genetex, USA), by Qdot 625 Western Blot Kit (Thermo Fisher Scientific).

2.2. Cloning, expression, characterization and production of P. pastoris based HPV L1 vaccine candidates

The yeast-codon optimized HPV16 L1 and HPV18 L1 transgenes were assembled by *de novo* synthesis (GeneArt™, Thermo Fisher Scientific), and cloned by *BstB*I/*Not*I (New England Biolabs, Ipswich, MA, USA) into the pPICZα expression vector (Thermo Fisher Scientific) under the control of the AOX1 promoter. The methods used for cloning were similar to those described by Sambrook and Russell [28]. The obtained pPICZ-HPV16L1 and pPICZ-HPV18L1 plasmids, propagated in *E. coli* and selected by Zeocin (25 μg/mL, Thermo Fisher Scientific) resistance, were linearized by *Sac*I digestion and used to transform electrocompetent *P. pastoris* KM71 by

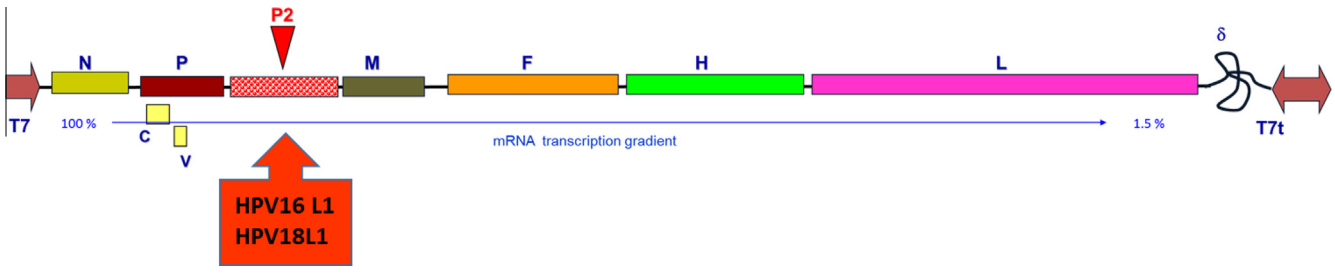

Fig. 1. Schematic representation of rMVEZ antigenome. N: nucleocapsid protein, P: phosphoprotein, M: matrix protein, F: fusion protein, H: attachment protein, L: large RNA-dependent RNA polymerase protein, C and V represents nonstructural proteins encoded in the P transcription unit. P2 represents position in measles vector to insert HPV16L1 or HPV18L1 genes as additional transcription unit.

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