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Heterologous papillomavirus virus-like particles and human papillomavirus virus-like particle immune complexes activate human Langerhans cells

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Abstract

Chimeric human papillomavirus virus-like particles (HPV cVLP) are currently being explored as a therapeutic vaccination strategy against cervical cancer. HPV cVLP are being explored as a result of their interaction with and activation of dendritic cells, a potent antigen-presenting cell. However Langerhans cells, another type of antigen-presenting cell, can interact with HPV cVLP especially during mucosal routes of vaccine administration. Langerhans cells are not activated by HPV cVLP, utilize a different endocytosis mechanism than DC for HPV cVLP uptake, do not initiate an immune response toward HPV cVLP derived antigens, and are potentially immunosuppressive after interaction with HPV cVLP. Taken together, these findings indicate that the overall effectiveness of HPV cVLP as a therapeutic vaccine may be reduced. Bovine papillomavirus (BPV) VLP, cotton-tail rabbit papillomavirus (CRPV) VLP, and HPV VLP immune complexes (IC), which are taken up via similar endocytosis mechanisms in DC and LC, activate both cell types. DC and LC incubated with these VLP upregulate surface activation markers and increase secretion of IL-12 p70. The activated cells are then able to initiate an immune response against chimeric VLP-derived antigens. These data indicate that other therapeutic vaccination strategies based on using chimeric BPV VLP, chimeric CRPV VLP, or chimeric HPV VLP immune complexes may be more effective in generating an immune response against HPV-induced diseases such as cervical cancer.

Keywords: Human papillomavirus; Vaccine; Antigen-presenting cell

1. Introduction

Human papillomavirus (HPV) type-16 infection of the upper layer of cervical mucosa is strongly linked to the generation of cervical cancer in women [1]. Although the majority of HPV-induced lesions are cleared, the time for clearance ranges from months to years. Some women do not initiate an effective immune response against HPV at all [2–4]. An HPV vaccine would greatly contribute to the health of women worldwide; however, HPV cannot be easily produced in vitro hindering vaccine development. One strategy currently being explored as a prophylactic vaccine, with promising results, is based on HPV virus-like particles (VLP) comprised of one of the HPV capsid proteins, L1, excluding all of the virus's genomic material [5]. This vaccine initiates potent virus-neutralizing antibodies capable of preventing high-risk HPV infection in vaccinated women without a fear of vaccine-induced transformation. This prophylactic vaccine, composed of only the L1 protein of HPV, will not be effective at providing therapy for patients previously infected with HPV. A therapeutic vaccine that ini-

Abbreviations: HPV, human papillomavirus; BPV, bovine papillomavirus; CRPV, cotton-tail rabbit papillomavirus; cVLP, chimeric virus-like particle; DC, dendritic cell; LC, Langerhans cell; APC, antigen-presenting cell; IC, immune complex; MHC, major histocompatibility complex; CTL, cytotoxic T lymphocyte

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tiates potent HPV E6 or E7 specific CTL is required as these are the only genes consistently expressed in cervical cancers [6,7].

There are no effective therapeutic HPV vaccines currently available. One therapeutic vaccine strategy that is explored utilizes chimeric VLP (cVLP) [8–10]. cVLP comprised of a fusion of HPV16-E7 to the L1 or L2 capsid proteins have been shown to initiate a potent E7-specific cytotoxic T cell (CTL) response in vaccinated mice when administered through the mouse's thin epidermal skin layer in the absence of adjuvant. The vaccine was found to be sufficient to protect mice against challenge with an E7-containing tumor cell line [11–13]. The reason behind this vaccine's high immunogenicity is a result of the cVLP's interaction with potent antigen-presenting cells (APC), dendritic cells (DC), residing at or near the vaccination site. DC are activated by cVLP and after the DC's interaction with naïve $CD8^+$ T cells are able to induce an efficient antigen-specific immune response [14,15].

A vaccine administered into human skin will interact with a high concentration of professional APC, such as DC and Langerhans cells (LC). LC form a 3-dimensional network in the epidermis of skin, whereas DC reside below the basal layer of the epidermis in the dermis [16]. These DC and LC endocytose antigen in the periphery, and depending on the nature of that antigen, will become activated, migrate to regional lymph nodes, and interact with T cells for the initiation of an antigen-specific immune response [17,18]. Therefore, administration of a vaccine into the skin may be an optimal route for a therapeutic vaccine. However, it is critical to determine if the resident APC are activated by that vaccine. If they are not activated, tolerance to antigens harbored within the vaccine may occur. Tolerance would occur if APC with peptide-MHC complexes and lacking co-stimulatory molecules on their surface interact with antigen-specific naïve T cells. These T cells will not receive the second signal required for proliferation and may become tolerized to the antigen presented by the APC.

We previously found that both human DC and LC internalize similar amounts of HPV cVLP, albeit through different endocytosis mechanisms [19,20]. DC were found to be activated after encounter with HPV cVLP, but LC were not [20]. DC, and not LC, were able to upregulate cell surface activation markers, increase secretion of IL-12 p70, and were able to induce an antigen-specific immune response in naïve $CD8^+$ T cells. The data indicated that LC were able to cross-present cVLP-derived antigens on their surface, however they did so in the absence of co-stimulation [19]. If LC that have taken up HPV cVLP do not become activated prior to their encounter with HPV specific T cells, those T cells will not proliferate and initiate antigen-specific immunity. Therefore, human LC may be immunosuppressive after encounter with HPV cVLP reducing the overall effectiveness of a therapeutic HPV cVLP vaccine. In the present study, we sought to determine if another VLP-based therapeutic vaccine approach would be able to activate both DC and LC thereby decreasing the possibility of immunosuppressive interactions. We found that two heterologous VLP, bovine papillomavirus (BPV) L1L2-HPV16-E7 cVLP and cotton-tail rabbit papillomavirus (CRPV) L1L2-HPV16-E7 cVLP, and HPV16 L1L2-E7 cVLP immune complexes (IC) were able to activate both DC and LC. After encounter with BPV cVLP, CRPV cVLP, and HPV cVLP IC, both DC and LC upregulated surface activation marker expression, increased IL-12 p70 and IL-15 secretion, and were able to induce an antigen-specific immune response to cVLP-derived antigens. In addition, BPV VLP, CRPV VLP, and HPV VLP IC were endocytosed via similar endocytosis mechanisms illustrating the importance of the endocytosis mechanism in the generation of an immune response. Taken together, the data indicate that BPV cVLP, CRPV cVLP, and HPV cVLP IC render both DC and LC capable of initiating a potent immune response against VLP-derived antigens. Therefore, BPV cVLP, CRPV cVLP, and HPV cVLP IC may serve as better overall therapeutic vaccine candidates against HPV-induced lesions than HPV cVLP.

2. Materials and methods

2.1. Antibodies and reagents

Monoclonal anti-HPV16 L1 antibody and antibodies to human CD80-FITC, CD86-FITC, HLA-DR, DQ, DP-FITC, and isotype controls, were purchased from BD PharMingen (San Diego, CA). HLA-A, B C-PE was purchased from DAKO (Glostrup, Denmark). Antibodies for flow cytometric analysis were used at 1:50 dilutions in PBS containing 1% FCS and 0.01% NaN_3. The recombinant human (rhu)-IL-4 and rhu-GM-CSF were purchased from Intergen (Purchase, NY) and stored according to manufacturers' instructions. Human TGF-β_1 was purchased from PeproTech (Rocky Hill, NJ) and stored according to manufacturers' instructions. LPS (*Escherichia coli* 026:B6) was purchased from Sigma (St. Louis, MO).

2.2. Virus-like particles and VLP immune complexes

HPV16-L1L2-HPV16-E7 cVLP, BPV-L1L2-HPV16-E7 cVLP, and CRPV-L1L2-HPV16-E7 cVLP were produced in insect cells and purified by sucrose and cesium chloride ultra-centrifugation as described [12]. VLP were tested by transmission electron microscopy for the presence of intact particles [21]. HPV cVLP IC were produced by incubating 10 μg HPV cVLP and 5 μg anti-HPV16-L1 antibody (clone H16.V5) for 30 min at room temperature. A Limulus assay (SIGMA) was used to detect and semi-quantitate endotoxin in the preparations. The endotoxin level in the preparations was found to be less than 0.085 EU/10 μg VLP and this level did not activate DC or LC (data not shown). Baculovirus DNA was not detected in the preparations as assessed by PCR (data not shown).