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Topically applied virus-like particles containing HIV-1 Pr55^{gag} protein reach skin antigen-presenting cells after mild skin barrier disruption

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ABSTRACT

Loading of antigen on particles as well as the choice of skin as target organ for vaccination were independently described as effective dose-sparing strategies for vaccination. Combining these two strategies, sufficient antigen recognition may be achievable via the transcutaneous route even with minimal-invasive tools. Here, we investigated the skin penetration and cellular uptake of topically administered virus-like particles (VLPs), composed of the HIV-1 precursor protein Pr55^{gag}, as well as the migratory activity of skin antigen-presenting cells (APCs). We compared VLP administration on ex vivo human skin pre-treated with cyanoacrylate tape stripping (CSSS, minimal-invasive) to administration by skin pricking and intradermal injection (invasive). CSSS as well as pricking treatments resulted in penetration of VLPs in the viable skin layers. Electron microscopy confirmed that at least part of VLPs remained intact during the penetration process. Flow cytometry of epidermal, dermal, and HLA-DR$^+$ APCs harvested from culture media of skin explants cultivated at air-liquid interface revealed that a number of cells had taken-up VLPs. Similar results were found between invasive and minimal-invasive VLP application methods. CSSS pre-treatment was associated with significantly increased levels of IL-1α levels in cell culture media as compared to untreated and pricked skin. Our findings provide first evidence for effective cellular uptake of VLPs after dermal application and indicate that even mild physical barrier disruption, as induced by CSSS, provides stimulatory signals that enable the activation of APCs and uptake of large antigenic material.

1. Introduction

New skin vaccination strategies are increasingly explored. A non-invasive patch application of vaccine may help overcome practical challenges associated with conventional injections [1]. In addition, the particular vaccine entry route may induce different qualities of immune responses [2]. Langerhans cells (LCs) are potent activators of antigen specific CD8$^+$ T cells, which can promote strong cellular immune responses [3]. A preferential induction of CD8$^+$ T responses is increasingly being pursued not only in the field of vaccination against intracellular pathogens, but also in the field of tumor vaccination [4]. In addition, approaches to target dendritic cell (DCs) are currently being explored to develop new generation vaccines and for the treatment of autoimmune diseases or allergic reactions [5–7]. Differential targeting of dendritic cell subsets may greatly improve skin vaccination efficacy [8]. Furthermore, combinations of different approaches, e.g., prime-boost regimen, may help mount complex immune responses required to fight chronic viral diseases or cancer [9].

Despite the fact that effective vaccine delivery across the skin barrier remains a challenge, the accessibility of the skin surface strongly

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encourages further exploration of non or minimally invasive vaccination methods. We recently introduced cyanoacrylate skin surface stripping (CSSS) as promising minimal-invasive method to enhance skin penetration and cellular uptake for topically applied nanoparticles [10,11]. This procedure has already been proved as safe for humans in in vivo studies for transcutaneous delivery of influenza vaccine [12,13]. In addition, CSSS induced the activation of LCs in human skin [11], whereas mild disruption of stratum corneum by tape stripping enhanced antigen up-take by LCs in mouse skin [14]. Just recently, a clinical trial using this delivery approach in combination with an anti-HIV DNA vaccine has been completed and results show that delivery of the vaccine across the skin shifted the immune reaction towards responses associated preferentially with mucosal and epidermal protection [15]. Such approaches offer new perspectives for differential shaping of desired cellular immunity required to fight the wide range of complex and diverse infectious diseases as well as cancers. Vaccine delivery in a particle-bound form may stabilize vaccine compounds sensitive to degradation, provide retention in the skin for optimal uptake by antigen-presenting cells (APCs) and, thus, increase immunogenicity. Biodegradable carrier architectures are of special interest, yet carrier stability and release properties may be not satisfactory in the tissue environment and change significantly from those measured in vitro. For example, destabilization of the carriers on the skin surface or in the skin before cellular uptake [16,17] may result in release of the antigen into the tissue which could be deleterious for the immunization success [18].

With this respect, virus-like particles (VLPs) represent highly promising carrier systems as they allow for simultaneous incorporation of multiple antigens into the capsid shell. In previous studies, VLPs induced efficient and effective cell-mediated immunity [19–24]. Systemic and mucosal antibodies are generated in response to VLP immunization and the immunogenicity of these vaccine candidates has been demonstrated using various routes of delivery [25,26]. VLP-based vaccines against hepatitis and human papilloma virus are being assessed in clinical trials [22,27].

In this study, we investigated the administration of VLPs to ex vivo human skin via the transcutaneous route using CSSS and pricking to enhance skin permeability and induce immune cell activation. The investigated VLPs are made of $Pr55^{gag}$ polyproteins, which assembly at the plasma membrane of the producer cells and bud in form of enveloped VLPs [23]. The $Pr55^{gag}$ precursor protein carries multiple HIV-1 epitopes comprising the major virus structural proteins (the p17 matrix protein, the p24 capsid protein, the p7 nucleocapsid protein, and the late domain-containing protein p6). These VLPs were shown to raise strong antibody responses in rabbits when injected intramuscularly [28]. In this study, we explored the possibility of using the transcutaneous route for VLP administration. Skin penetration, cellular uptake, migration of DCs as well as representative inflammatory cytokines were determined to evaluate the effects of different skin pre-treatments on skin environment and VLP interaction with skin barrier and immune cells.

2. Methods

2.1. Production and characterization of fluorescently labeled VLPs

A DNA plasmid with chimeric genes encoding for $Pr55^{gag}$ was transferred to baculovirus *Autographa californica* nuclear polyhedrosis virus (AcNPV) according to standard procedures described by Wagner et al. [29]. The recombinant viruses were propagated on *Spodoptera frugiperda* (SF9) cells in TC100 medium (Gibco BRL, Eggenstein, Germany) supplemented with 10% fetal calf serum (FCS), 100 IU/mL penicillin and 100 μg/mL of streptomycin [30]. For large scale preparation, the recombinant viruses were let to infect HighFive™ insect cells derived from *Trichoplusia ni* egg cell homogenates (Invitrogen Inc., San Diego, CA, USA) [28,31]. The insect cells were let to proliferate in serum-free Insect-Xpress medium (Cambrex, Walkersville, MD, USA) supplemented with 100 μg/mL kanamycin sulfate (PAN, Oberasbach, Germany) at 24–27 °C [31]. The insect cells expressed the chimeric proteins and secreted them in form of VLPs into the culture medium. After 3 days, cell culture supernatants were precleared by centrifugation (2000 × *g*, 10 min, 4 °C) and pelleted by sedimentation through a 30% sucrose cushion (100,000 × *g*, 2.5 h, 16 °C) [28]. The VLPs were let re-suspend overnight in phosphate buffered saline (PBS, Biochrom, Berlin, Germany). Thereafter, they were incubated for 1 h at room temperature with 10 μM 5-carboxy-fluorescein diacetate (CFDA) succinimidyl ester (Molecular Probes, Eugene, OR, USA,) and let react with the primary amines of the VLP proteins. CFDA was useful to detect the intracellular uptake of VLPs by HaCaT keratinocytes, because its fluorescence increases when intracellular esterases cleave the acetate groups [32]. VLPs were further purified by separation on 10–50% sucrose gradients and subsequent dialysis of combined antigenic peak fractions against PBS [29]. Pooled fractions were analyzed using separation on a denaturing 12.5% sodium-dodecyl sulfate (SDS) polyacrylamide gel followed by Coomassie brilliant blue staining. The identity of the produced proteins was verified by immunoblotting. The proteins were transferred from SDS gels to nitrocellulose membranes (Millipore, Bedford, MA01730, USA) by electroblotting in a transfer buffer (25 mM Tris, 190 mM glycine, 20% methanol). The $Pr55^{gag}$ was quantified using a commercial p24-specific antigen sandwich assay (Abbott, USA). Purified VLPs were stained with uranyl acetate and imaged by transmission electron microscopy (TEM) using a Zeiss EM906 microscope at a voltage of 80 kV.

2.2. Tissue samples

Human skin explants (breast, abdomen, upper arm, face-lifting) obtained from healthy individuals, undergoing plastic surgery, were obtained after written informed consent within 6 h post-surgery. Experiments were run with the authorization of the Ethic committee of Charité - Universitätsmedizin Berlin (approval EA1/135/06, renewed on July 2015) and in accordance with the Declaration of Helsinki guidelines. Skin tissues with any macroscopic or microscopic injury were excluded from experiments. Subcutaneous fat was mostly removed.

2.3. Uptake of VLPs by isolated primary epidermis cells

Single cell suspensions of epidermis cells were prepared by digestion of human foreskin or adult human skin and Langerhans cells were enriched by magnetic cell separation (MACS, human CD1c-Dendritic Cell Isolation Kit, Miltenyi, Germany) as described elsewhere [10]. The separated $CD1c^+$ and $CD1c^-$ cell populations were incubated with VLPs (2 h, 50 μg/mL) in RPMI 1640 supplemented with 10% fetal calf serum, 100 U/mL penicillin and 100 μg/mL streptomycin. Cells were washed in PBS, stained with anti-CD1a-Apc antibody, and analyzed by flow cytometry (FACS Calibur, BD, Germany) and single cell microscopy using a confocal fluorescence microscope (CLSM, LSM Exciter, Zeiss, Germany). For electron microscopy analysis, cells were treated as described in Wagner et al. [28] and imaged with a Zeiss EM906 (Zeiss, Oberkochen, Germany) at a voltage of 80 kV.

2.4. Administration of VLPs

Three different administration procedures were evaluated: transcutaneous application after mild barrier disruption by CSSS, application by pricking, and intradermal (ID) injection. CSSS was performed on excised human skin as described elsewhere [10,16]. Briefly, after cleaning the skin surface with PBS, one droplet of cyanoacrylate super glue (UHU, Germany) was spread on each cm^2 of skin surface followed by application of an adhesive tape. The tape was removed after 20 min and the VLPs were applied on the selected area. The invasive approach