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# Epithelial stem cells as mucosal antigen-delivering cells: A novel AIDS vaccine approach

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ABSTRACT

A key obstacle limiting development of an effective AIDS vaccine is the inability to deliver antigen for a sufficient period of time resulting in weak and transient protection. HIV transmission occurs predominantly across mucosal surfaces; therefore, an ideal vaccine strategy would be to target HIV at mucosal entry sites to prevent infection. Such a novel strategy relies on the activation of mucosal immune response *via* presentation of viral antigens by the mucosal epithelial cells. The use of a terminally differentiated epithelial cell promoter to drive expression of antigens leading to viral protein production in the upper layers of the epithelium is central to the success of this approach. Our results show that when administered intradermally to mice, a GFP-reporter gene under the transcriptional control of the involucrin promoter is expressed in the upper layers of the epidermis and, although transduced cells were very low in number, high and sustained anti-GFP antibody production is observed *in vivo*. A subsequent experiment investigates the effectiveness of GFP-tagged replication-competent SIVdeltaNef and GFP-tagged replication-deficient SIVdeltaVifdeltaNef constructs under the transcriptional control of the involucrin promoter. Optimal conditions for production of pseudotyped VSV-G viral particles destined to transduce basal epithelial stem cells at the mucosal sites of entry of SIV in our animal model were determined. Altogether, the data demonstrate the feasibility of an epithelium-based vaccine containing involucrin-driven viral antigen encoding sequences that integrate into epithelial stem cells and show long-term expression in the upper layer of the epithelium even after multiple cycle of epithelia renewal. Such epithelium-based vaccine should elicit a long-term immunity against HIV/SIV infection at the site of entry of the virus.


## 1. Introduction

Despite recent advances in developing immunogenic-induced HIV/AIDS vaccines [1], achieving protection against HIV infection remains elusive. The HIV virus presents barriers to effective immunity including antigenic variability; resistance to neutralizing antibodies; down regulation of MHC class I and CD4 on infected cells; and, preferential destruction of viral-specific $CD4^+$ lymphocytes [2,3].

Developing an effective vaccine restricting viral replication at the mucosal portal of entry remains an important goal since HIV transmission occurs predominantly across genital and rectal mucosal surfaces. The presence of HIV-specific T lymphocytes in the mucosa and at sites of early viral replication is an important factor for vaccine efficacy, as it would inhibit HIV spread into adjacent lymph nodes. Such approach requires (i) a life-long stimulation of the immune system with viral antigens providing a strong barrier to viral replication; and, (ii) a targeted immune response at sites of primary HIV replication (vaginal or rectal mucosa). Research using attenuated Simian Immunodeficiency Virus (SIV) in macaques often accomplishes effective and durable protection against pathogenic SIV challenge. One promising approach utilizes a replication-defective SIV limited to a single cycle of infection [4–7].

*Abbreviations:* AIDS, acquired immunodeficiency syndrome; BD, Becton Dickinson immunocytometry systems; Bp, base pair; CMV, cytomegalovirus; CTL, cytotoxic T lymphocytes; DNA, deoxyribonucleic acid; HIV, human immunodeficiency virus; INV, involucrin; K, keratin; MMP-9, matrix metalloproteinase-9; MID, monkey infectious dose; MVA, modified vaccinia ankara; pCMV, CMV promoter; pINV, involucrin promoter; SIV, simian immunodeficiency virus; US, upstream sequence; VSV-G, vesicular stomatitis virus G glycoprotein.

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This manuscript details a novel vaccine approach based on the ability of therapeutic lentiviral vectors integrated in epidermal or mucosal epithelial stem cells to induce virus-specific cellular immune responses at mucosal sites of viral entry. The strategy employs the well-characterized keratinocyte-specific involucrin locus (INV) promoter that ensures tissue specific expression in terminally differentiated epithelial cells [8]. By replacing viral enhancer elements with the minimal INV promoter, both tissue-specific expression and high viral titers can be achieved.

We have developed GFP-tagged replication-competent SIVmacdeltaNef and replication-deficient SIVmacdeltaVifdeltaNef constructs under the transcriptional control of the involucrin promoter (pINV).

The mechanisms controlling the involucrin transcription in the epidermis and mucosa are well conserved, as the human pINV is active in mouse epidermis and vaginal/ectocervix epithelium. This allows the use of a mouse model to develop the preliminary groundwork for our vaccine approach. We anticipated that pINV would drive expression of SIV-derived constructs in terminally differentiated epithelial cells. Long-term antigen expression in the epithelium upper layers will occur even after multiple epithelia renewal cycles, thus eliciting a long-term immunity against HIV/SIV infection at viral entry sites. We show that the involucrin-driven lentiviral vector is exclusively expressed in the epithelium upper layer, and mediates long-lasting antibody production in mice. We also demonstrate that an involucrin-driven SIV is preferentially expressed in human calcium-differentiated keratinocytes.

## 2. Materials and methods

### 2.1. Construction designs

Recombinant DNA plasmids and vaccine vectors were constructed using restriction endonucleases and, ligations performed using Ligafast Rapid DNA Ligation protocol (New England Biolabs). OneShot TOP10 Chemically Competent *E. coli* (Invitrogen) were used for plasmid DNA amplification. Bacteria were routinely grown in Luria Broth (LB) supplemented with ampicillin (final concentration: 100 μg/ml). Plasmid DNA isolation was achieved using EndoFree Plasmid Mega Kits (Qiagen).

#### 2.1.1. Construction of Involucrin promoter-driven vectors

The minimal involucrin promoter [10] was synthesized by overlapping PCR and replaced the pPGK promoter in vector pRRL.SIN.cPPT.pPGK-GFP.WPRE [40], using Cla-I/BamH-I endonuclease restriction and subsequent ligation, kindly provided by Dr Trono (EPFL, Swissland).

#### 2.1.2. SIV vaccine construction

The IRES-GFP fragment from pBlueScript IRES-GFP plasmid (Invitrogen) was amplified by PCR using specific primers containing XhoI restriction sites and cloned into the SIVmac239megalo3′ plasmid [35] between positions 9500 and 9690 to generate plasmid pSIVmac239megalo3′/IRES-GFP. The *nef* gene in plasmids pSIVmac239megalo5′ and pSIVmac239megalo3′/IRES-GFP was deleted and replaced by inserting the STR fragment from pSIVmac239/STR plasmid [41] between the EcoR-I/Not-I or Not-I/Nhe-I restriction sites, respectively, giving rise to pSIVmac239megalo/STR5′ and pSIVmac239megalo/STR3′/IRES-GFP.

To avoid the TAR/Tat transcriptional control, we inactivated TAR sequence by homology to HIV using the following primers: 5′-GCGGCCGCTGCGCAGAGGCAGAAAGAGCCATTGGAGGTTCTCTCCA-GCACTAGC and 5′-AGGAGGAGCATTGGTGTTCCCTGCTAGACTCTC-ACC. This fragment was subcloned and introduced at the Fsp-I and Nar-I sites of plasmids pSIVmac239megalo/STR5′ and pSIVmac239megalo/STR3′/IRES-GFP creating plasmids pSIVmegaloSTR5′/TAR* and pSIVmegaloSTR3′IRES-GFP/TAR*, respectively. A full-length construct was reconstituted after ligation of its 5′- and 3′- halves together. The resulting ubiquitously transcriptionally regulated construct was referred to as pCMV-IE/SIV/deltaNef/IRES-GFP.

The 570 bp involucrin promoter was cloned in place of the 5′-CMV promoter of pSIVmac239megalo5′ (NotI/FspI restriction sites) and pSIVmac239megalo3′ (NotI/FspI restriction sites) plasmids. A full-length epithelia-specific transcriptionally regulated construct was reconstituted after ligation of its 5′- and 3′- halves (named: pInv/SIV/deltaNef/IRES-GFP).

To obtain replication-deficient viral constructs, the *vif* genes from the 5′ moiety of pCMV-IE/SIV/deltaNef/IRES-GFP and pInv/SIV/delatNef/IRES-GFP plasmids (pSP72 backbone) were deleted by replacing the PacI/SphI fragment with the PacI/SphI fragment of pSIVdeltaVif5′, kindly provided by Dr. Desrosiers [42]. The resulting recombinant plasmids were named pCMV/SIV5′/deltaVif and pInv/SIV5′/deltaVif.

Full-length constructs were obtained by ligation of the 3′ moiety of pCMV-IE/SIV/deltaNef/IRES-GFP or pInv/SIV/deltaNef/IRES-GFP plasmids (SphI/EcoRI). The full-length replication-deficient viral constructs were named pCMV-SIV (pCV/SIV/deltaVif/deltaNef/IRES-GFP) and pINV-SIV (pInv/SIV/deltaVif/deltaNef/IRES-GFP).

### 2.2. CFA immunization and viral transduction of mice epidermis

Mice were immunized by footpad subcutaneous injection of emulsified Complete Freund's Adjuvant (CFA) with 200 μg of His-tagged purified GFP in PBS (50/50; v/v). Viral transduction was performed as previously described [43]. Briefly, FVB mouse shaved backs were dermabraded using a felt wheel. Day 3 post-abrasion, animals were inoculated with 50 μl (10e8 pfu) of: VSV-pseudotyped pRRL.SIN.cPPT.pINV-GFP.WPRE; pRRL.SIN.cPPT.pPGK-GFP.WPRE (heat inactivated, 30 min at 56 °C); pRRL.SIN.cPPT.pINV-GFP.WPRE; or control pRRL.SIN.cPPT.pPGK-GFP.WPRE viruses.

### 2.3. Histological analysis

Day 7 post-inoculation, mice were sacrificed. The inoculation site was snap frozen in OCT compound. Eight μm-cryosections were fixed (10 min, 4% paraformaldehyde), rinsed (PBS) and examined by fluorescent microscopy.

### 2.4. Anti-GFP antibody quantification in mice serum

The amount of anti-GFP antibody in mice serum was determined by an in-house ELISA using recombinant GFP protein [44] coated on maxisorp plates (Nalge Nunc, Rochester, USA) in a 1.5 mM carbonate/bicarbonate pH = 9.6 buffer. The serum was diluted 100- to -500-fold and incubated (1 h, room temperature) in a 1.5 mM carbonate/bicarbonate buffer. Anti-GFP immunoglobulins were quantitated after incubation (1 h, room temperature) with goat anti-mouse Ig kappa light chain-HRP (Abcam, USA), and subsequent color development.

### 2.5. Human keratinocytes differentiation

Normal Human Epidermal Keratinocytes (NHEK, PromoCell, Germany) were cultured in keratinocyte growth medium 2 (PromoCell, Germany), according to manufacturer's instructions (Merck Millipore, Germany). For NHEK terminal differentiation, we used

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