Mucosal HIV vaccine Targeting Host Epithelial Stem Cells for Long-Term Immunity

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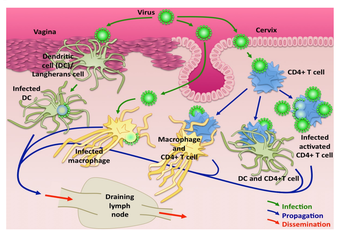
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Vaccine Construct and Strategy

A key obstacle to an effective AIDS vaccine is the **inability to deliver antigen for a sufficient period of time leading to weak and transient protection.** Because HIV transmission occurs predominantly **across mucosal surfaces**, the ideal vaccine strategy would be **to target HIV at mucosal entry sites to prevent infection.**

The epithelial barriers in the genital tract are protected by innate immune factors in the mucus covering the epithelium. Some of these factors are present within the multi-stratified epithelial layer constituting a second line of defense.



We developed a SIV single-cycle vaccine under the control of the involucrin promoter (pINV-SIVsc), which was tested for its ability to drive SIV expression in terminally-differentiated epithelial cells, induce mucosal immune responses and protection against SIV. **We are using the epithelial stem cells as permanent/recurrent source of viral antigens and their differentiated offspring as antigen-producing presenting cell**

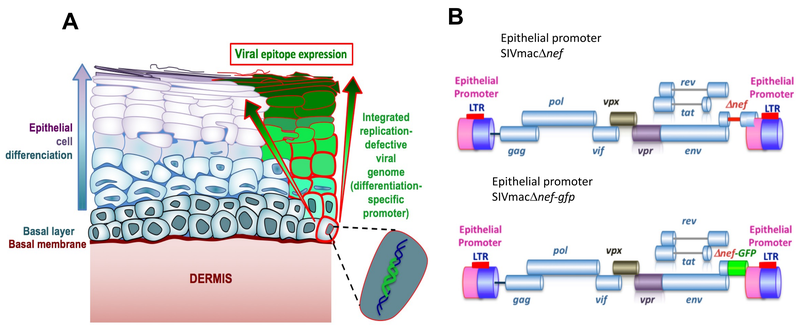


Figure.1 Schematic representation of the vaccine approach and Vaccine constructs. (A) After integration of the involucrin-driven viral constructs into the basal layer stem cells, the cells divide and differentiate triggering SIV antigens expression in the epidermis upper corneal layers. The right panel shows the level of SIV antigens expression from the basal stem cells layer to the differentiated epidermal cells of the epithelia *stratum corneum*. The arrows depict the orientation of the cells differentiation. SIV antigens expression is transcriptionally regulated by the involucrin promoter; **(B) Genetic map of the pINV-SIVsc vaccine** (ΔTARΔnef) and pINV-SIVsc-GFP (ΔTARΔnef-GFP).

During the vaccine phase:
i. No side effect was noted; ii. No cell-associated nor cell-free virus was detected in peripheral blood mononuclear cells (PBMCs) isolated from blood; iii. No replicating virus was recovered from blood, vaginal or rectal tissues. In addition, PBMCs from vaccinated animals were co-cultured with freshly isolated and activated PBMCs from naive unvaccinated animals, yielding no virus replication.

Methods and Vaccine Expression

Twelve female macaques were immunized intravaginal (1 dose, atraumatic, needleless) at week 0 and monitored for specific immune responses in blood, mucosal secretions, various lymphoid/non-lymphoid tissues. Eight animals were challenged with repeated intravaginal low-doses SIVmac239 (2500 $TCID_{50}$/ml) at weeks 12 or 24. Eight additional females served as unvaccinated SIV-infected Controls.

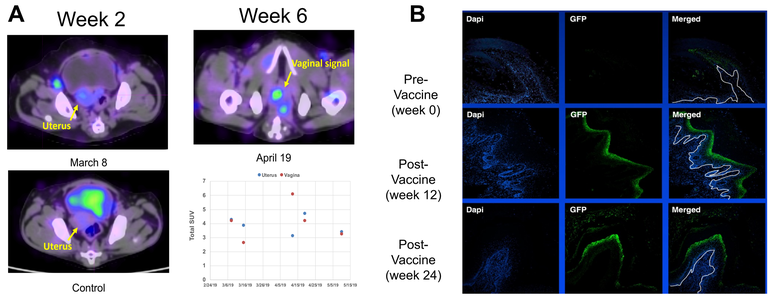
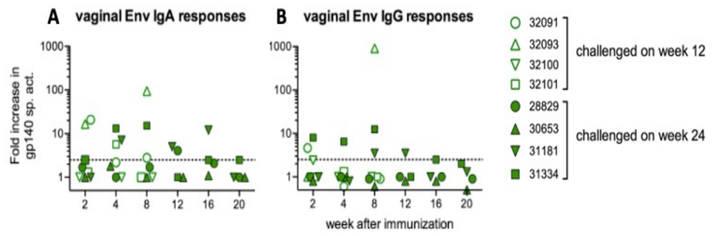


Figure 2: The vaccine expression: (A) Imaging the expression of SIVenv using p7D3-Cu^{64} based immunoPET/CT detection and quantification of signal in female reproductive tract and draining lymph node over several weeks post immunization: The female was vaccinated once (intravaginal, atraumatic "Drop") with pINV-SIVsc. Signal quantification by SUVtot shows variation of vaginal expression with various phases of the reproductive cycle; **(B) Vaccine expression profiles by immuno- histofluorescence of the vaginal tissues** (dotted line indicates the localization of the basal layer).

Results Post-Vaccine



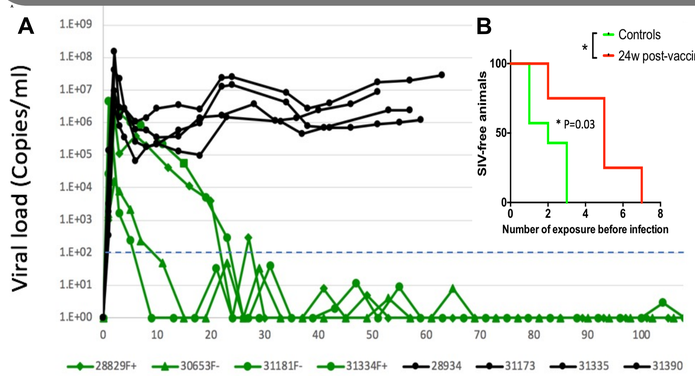
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% Gag Tetramer$^+$ CD8$^+$ CD3$^+$ T lymphocytes*

Macaques	Blood	Spleen	Ing LN	Vagina**	Cervix**	Ileum***	JEJ***	Colon***	Rectal
pINVscSIV (females, n=4)									
Week 0 (n=4)	0.01/0.00	n/d	0.00/0.02	0.01/0.03	0.04/0.02	n/d	n/d	n/d	0.01/0.03
Week 8 (n=2)	0.17/0.19	0.11/0.09	0.14/0.28	0.72/0.67	0.40/0.53	0.22/0.19	0.21/0.15	0.28/0.13	n/d
Week 24 (n=2)	0.48/0.36	0.35/0.41	0.34/0.62	1.24/1.03	0.83/0.98	0.35/0.51	0.38/0.62	0.32/0.39	n/d

Figure 3: SIV-specific immune responses in vaccinated female maca ques. Vaginal Env IgA (a) and Env IgG (b)anti-gp140 antibody specific tite rs were detected 12 and 24 weeks post pINV-SIVsc. Strong SIV Gag-specific CD8+ T-cell responses (C) were detected in various lymphoid and non-lymphoid tissues.

Results SIV Post-Challenged



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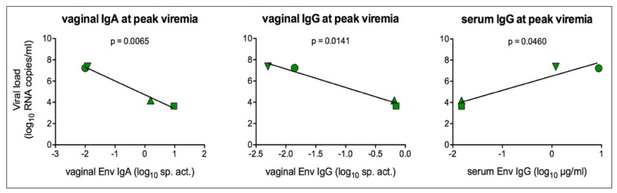


Figure 6: (A) Viremia following challenge in 24-week vaccinated Females; (B) Number of challenges required to achieve infection 24-weeks post-vaccine with pINV-SIVsc; (C) Correlation between anti-gp120 antibody titers in the vaginal secretions and peak viremia.

Summary

The pINV-SIVsc vaccine drives SIV antigen expression in differentiating epithelial cells and:
- -does not disseminate into the blood
- -induces strong mucosal antibodies (IgA, IgG) against SIV Env
- -induces strong mucosal CD8+ T cells against SIV Gag
- -Is a safe strategy compared to traditional live attenuated vaccines
- -does not induce detectable long-term side effects (>3 years)

Repeated low-dose challenges revealed:
- -a significant delay in vaginal viral transmission (p=0.03)
- -a significant control of viremia post-SIV transmission in vaccinated females compared to unvaccinated controls, such as: i. 2-3 log-reduction at peak; ii. 4-5 log-reduction at set-point; and, iii. undetectable viremia as early as 8 to 22 weeks post-SIV

The study demonstrated the efficacy of an epithelial stem cell-based vaccine to serve as antigen delivery to generate specific mucosal antibody and cellular immune responses leading to a significant delay in infection followed by rapid and durable viral control to undetectable.

NIAID R56 AI084171, R01 AI090705, R01 AI172539
U.S. Patents: 9730996, 2017; 10751406, 2020